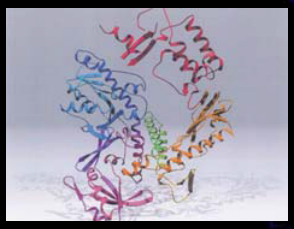
TOYOBO
Ideas & Chemistry

TOYOBO
Life Science

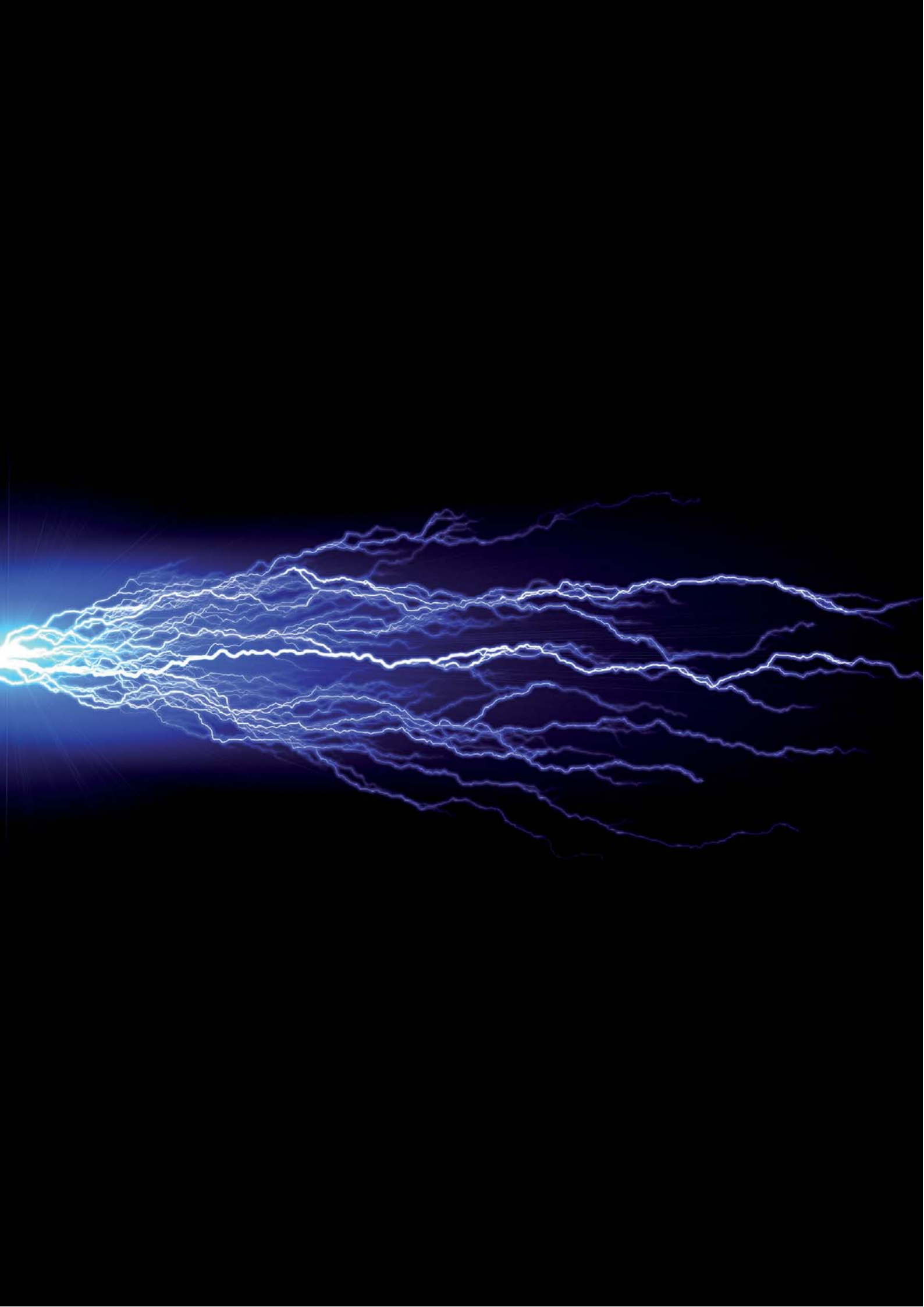
2014 / 2015






- PCR enzymes & kits
- Real-time PCR Master Mix
- Reverse transcriptase & kits
- PCR related products
- Modifying enzymes, Cloning & Mutagenesis
- Reaction accelerators

Axil Scientific
YOUR RESEARCH IS OUR BUSINESS



CONTENTS

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CONTENTS

1 PCR enzymes & kits

KOD -Plus- Neo (→ p 4)
KOD -Plus- Neo exhibits excellent PCR fidelity because of its efficient 3'→5' exonuclease activity (proof reading activity).

KOD FX Neo (→ p 8)
KOD FX Neo exhibits excellent PCR success on difficult targets and effective for amplification from crude specimens.

2 Real-time PCR Master Mix

THUNDERBIRD®qPCR Mix series (→ p 16)
This reagent is a highly efficient 2x master mix for real-time PCR using Taq DNA polymerase. TaqMan® probes and SYBR® Green I versions are available.

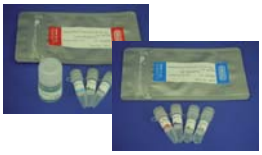
3 Reverse transcriptase & kits

KOD SYBR®qPCR Mix (→ p 18)
This reagent is a highly efficient 2x master mix for real-time PCR using KOD exo(-) DNA Polymerase and SYBR® Green I.

4 PCR related products

ReverTra Ace®qPCR RT Master Mix with gDNA Remover (→ p 24)
This kit includes master mix reagents for reverse transcription and the removal of genomic DNA [DNase I treatment].

5 Modifying enzymes, Cloning & Mutagenesis



***SuperPrep*® Cell Lysis & RT Kit for qPCR** (→ p 25)

This kit contains lysis reagents for the preparation of cell lysates containing RNAs that can be used as templates for reverse transcription and reverse transcripton ragents for cDNA synthesis from the crude cell lysates.

6 Reaction acceralators

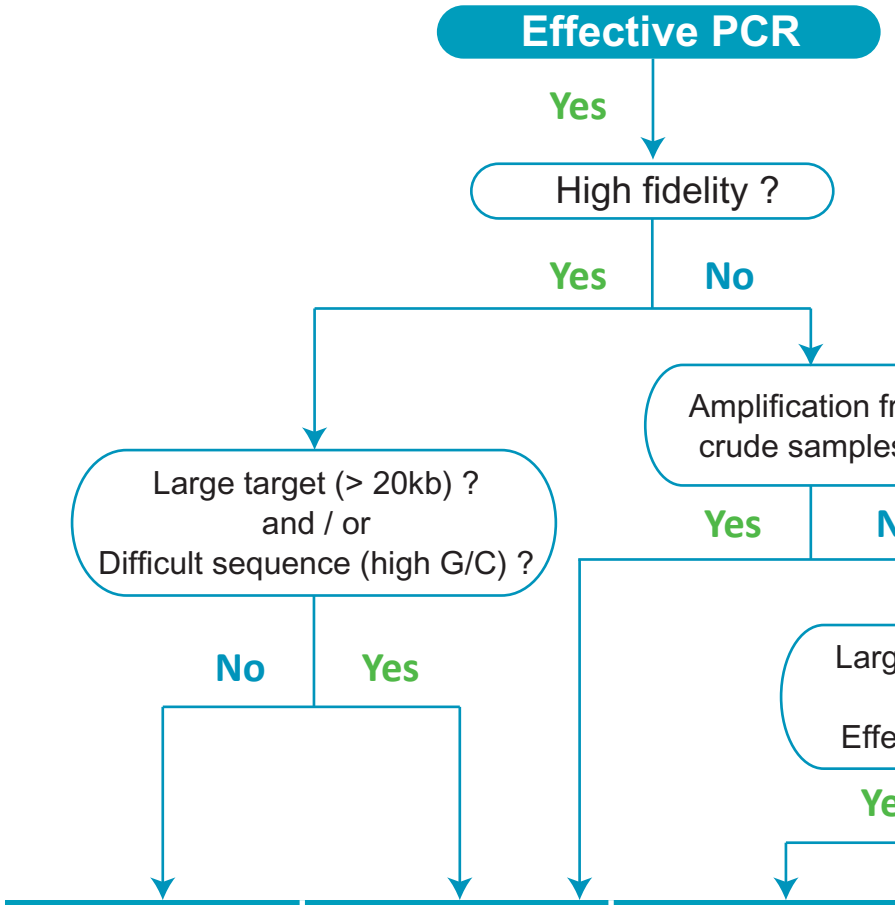
KOD -Plus- Mutagenesis Kit (→ p 30)

This kit is an inverse PCR (iPCR)-based site-directed mutagenesis kit using KOD DNA polymerase as a high-fidelity PCR enzyme.

***Can Get Signal*® series** (→ p 34)

Can Get Signal® series improves sensitivity, specificity, and signal-to-noise ratio (S/N) for western blot, dot blot, enzyme-linked immunosorbent assays (ELISA) and immunohistochemistry.

Quick Selection Guide for effective PCR



KOD -Plus- series	KOD FX series	Blend Taq® series	Quick Taq® HS DyeMix
Fidelity compared with Taq DNA polymerase (fold increase)			
80-fold	11-fold	3-fold	1-fold
-High fidelity PCR for Cloning -High Speed PCR (30 sec./kb)	-Crude samples (Blood, mouse tail, Plant etc.) -Microorganisms bearing cell walls (yeast, fungi etc.) -Difficult targets (Long > 20 kb, high GC content)	-General PCR -Long PCR -Colony PCR	-General PCR -Colony PCR -Premixed
			
p 3~5	p 6~9	p 11	p 12
Recommended TA cloning kits: TArget Clone™ series (p 29)			
TArget Clone™ -Plus-	TArget Clone™ -Plus-	TArget Clone™	TArget Clone™

Overview and PCR enzymes selection guide

Toyobo has various unique PCR reagents to choose from. The following table shows the characteristics of each product.

KOD DNA polymerase was isolated from the hyperthermophilic archaea *Thermococcus kodakaraensis* KOD1, and possesses superior fidelity and processivity that enables more accurate and efficient amplification that can be achieved with conventional PCR enzymes incruding Pfu and Taq DNA polymerases.

Table 1. Properties of thermostable DNA polymerases

Property	Value for indicated DNA polymerase		
	KOD DNA polymerase	Pfu DNA polymerase	Taq DNA polymerase
Origin	Archaea	Archaea	Bacteria
Thermostability (half-life)	95°C,12 h; 100°C,3.0 h	95°C, 6h; 100°C, 2.9h	95°C, 1.6 h
5'→3' exonuclease activity	-	-	+
3'→5' exonuclease activity	+	+	-
Processivity (bases)	>300	<20	ND
Elongation rate (bases/sec)	106-138	25	61

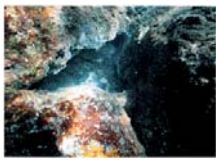


Fig. 1. Solfatara
(Kodakara island, Japan)

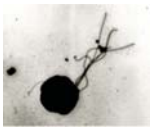


Fig. 2. *T. kodakaraensis* KOD1
This strain was isolated from a solfataric hot spring (Fig. 1).

The KOD -Plus- series was developed based on KOD DNA polymerase and exhibits high PCR fidelity. The KOD FX series was developed based on KOD DNA polymerase and shows a much greater PCR success-rate (based on efficiency and elongation capabilities) than Taq-based PCR enzymes. KOD FX is also effective for amplification from crude specimens (*e.g.* mouse tail lysate, plant lysate and organisms bearing cell walls (*e.g.* yeast, fungus, gram-positive bacteria). KOD -Plus- Neo and KOD FX Neo contain an elongation enhancer that enables long range and fast PCR. The Blend Taq® series and KOD Dash use a DNA polymerase mixture consisting of a DNA polymerase lacking a 3'→5' exonuclease (proofreading) activity [*e.g.* KOD exo(-) DNA polymerase and Taq DNA polymerase] and a small amount of an archaeal DNA polymerase with proofreading activity. Because the proofreading activity repairs mis-incorporated nucleotide bases, which cause termination of a polymerase reaction, PCR with this mixed enzyme solution enables highly efficient and sensitive amplification. Tth polymerase exhibits reverse transcriptase activity in addition to DNA polymerase activity in the presence of Mn^{2+} ions. Therefore, this enzyme enables "one-step RT-PCR" including reverse transcription and PCR steps.

Enzyme	Product Name	Fidelity (3'→5'Exonuclease activity)	Efficiency	Velocity (Extension time)	Target size	Amplification from Crude samples	Hot start	Reverse Transcriptase activity	PCR Product Ends	Application	Reference page
KOD DNA polymerase	KOD -Plus-	++++ [80 folds]	++	1 min/kb (Taq-like condition)	~12 kb	+	✓	-	blunt	Cloning	3
	KOD -Plus- Neo	++++ [80]	++	≦ 0.5 min/kb	~24 kb	+	✓	-	blunt	Cloning	4
	KOD FX	+++ [11]	+++	1 min/kb (Taq-like condition)	~24 kb	+++	✓	-	blunt	・Genotyping ・Cloning (High G/C)	6
	KOD FX Neo	+++ [11]	+++	≦ 0.5 min/kb	~40 kb	++++	✓	-	blunt	・Genotyping ・Cloning (High G/C)	8
	KOD Dash	+ [3]	+++	≦ 0.5 min/kb	~18 kb	++		-	mixed (blunt & 3'-dA)	・Colony PCR ・Detection	10
Taq DNA polymerase	rTaq DNA polymerase	- [1]	+	1 min/kb	~2 kb	+		-	3'-dA	・General PCR	13
	Quick Taq® HS	- [1]	+	1 min/kb	~4 kb	+	✓	-	3'-dA	・Colony PCR ・General PCR	12
	Blend Taq®	+ [3]	++	1 min/kb	~23 kb	++		-	mixed (blunt & 3'-dA)	・General PCR (Longer targets) ・Colony PCR ・Detection	11
	Blend Taq® -Plus-	+ [3]	++	1 min/kb	~23 kb	++	✓	-			
Tth DNA polymerase	rTth DNA polymerase	- [1]	+	1 min/kb	~2 kb	+		✓ (Mn^{2+})	3'-dA	・General PCR ・RT-PCR	13
	RT-PCR Quick Master Mix	- [1]	+	1 min/kb	~2 kb	+	✓	✓	3'-dA	・One-step RT-PCR	14

++++: Best, +++: Excellent or Strong, ++: Good or Moderate, +: satisfactory, -: Not good or minus
[ ] : Accuracy of each PCR enzyme (reagent) when the accuracy of Taq DNA polymerase is set to 1.

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KOD -Plus-

Code No. KOD-201 200 U
<200 reactions [50 µl per reaction]>

Store at -20 °C

Components:

KOD -Plus- (1.0 U/µl)*	200 µl
10 x PCR Buffer for KOD -Plus-	1 ml
25 mM $MgSO_4$	1 ml
2 mM dNTPs	1 ml

*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize polymerase and 3'→5' exonuclease activity for hot start technology.

KOD -Plus- is based on DNA polymerase from the hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1(1)(2). KOD -Plus- exhibits excellent high PCR fidelity and efficiency. The enzyme solution of KOD -Plus- contains two types of anti-KOD DNA polymerase antibodies that inhibit polymerase and 3'→5' exonuclease activity, thus allowing for Hot Start PCR(3). KOD -Plus- generates blunt-end PCR products, due to 3'→5' exonuclease (proof-reading) activity.

Features

- **The PCR error ratio of KOD -Plus- is approximately 80 times less than that of Taq DNA polymerase.**
- **Enables the following amplifications (maximum): 21 kb from phage lambda DNA, 12 kb from human genomic DNA, and 7 kb from cDNA.**
- **TArget Clone™ -Plus- [p29] can be applied to the TA cloning of blunt-end PCR products amplified using KOD -Plus-.**

Table Comparison of the mutation frequency of each PCR enzyme.

	Colony number		Mutation frequency (%)
	Total	Mutant	
KOD -Plus-	10,610	10	0.09
High fidelity PCR enzyme (A company)	10,900	68	062
Pfu based DNA polymerase	5,520	76	1.17
Taq DNA polymerase	10,560	780	7.39

*PCR fidelity was based on the mutation frequency of PCR products using a positive-selection base assay with the rpsL gene.

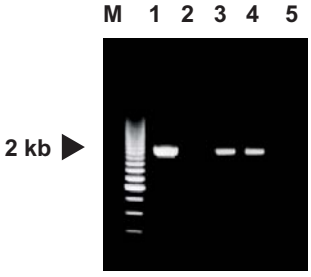
Applications

- High fidelity PCR

Application data

1. Amplification of a GC-rich target

The GC rich target, lipase gene (Pseudomonas sp.) [GC content = 70%], was amplified using various PCR enzymes. KOD -Plus- could amplify the target gene without additives such as DMSO or PCR enhancer.



1: KOD -Plus-
2: Taq DNA polymerase
3: Taq DNA polymerase + 5% DMSO
4: Taq DNA polymerase + PCR ehnancer (company A)
5: High efficient Taq DNA polymerase (company B)
M: 200 bp Ladder Markers

Target: Lipase gene (*Pseudomonas sp.*) [GC=70%]
Sample: Plasmid clone 10 ng / 50 µl reaction

[Cycling conditions of KOD -Plus-]

94°C, 2min.
98°C, 10sec.
60°C, 30sec.
68°C, 2 min.
30 cycles

References

1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.,* 63: 4504-10 (1997)
2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.,* 306: 469-77 (2001)
3) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem.,* 126: 762-8 (1999)

High fidelity & efficient & fast PCR enzyme

KOD -Plus- Neo

KOD -Plus- Neo is based on a DNA polymerase from the hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1(1) (2). This polymerase exhibits excellent PCR fidelity because of its efficient 3'→5' exonuclease activity (proof reading activity). This product contains a unique "elongation enhancer" that suppresses the "plateau effect" produced by conventional PCR. Therefore, this reagent exhibits greater amplification efficiency and elongation capability.
Moreover, this enzyme requires only 30 sec/kb for the PCR extension step. This facilitates the long range PCR.
This enzyme contains two anti-KOD DNA polymerase antibodies that inhibit polymerase and 3'→5' exonuclease activity, thus allowing for Hot Start PCR(3). This polymerase generates blunt-end PCR products due to 3'→5' exonuclease (proof-reading) activity.
KOD FX Neo is the improved version of KOD -Plus- (Code No. KOD-201).

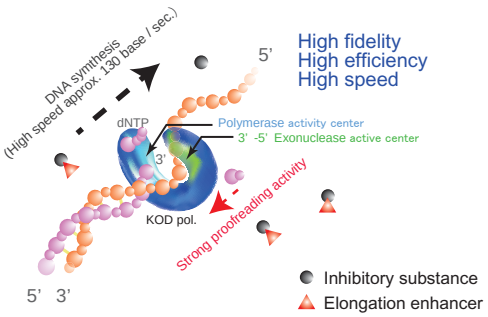
Code No. KOD-401 200 U
<200 reactions [50 μl per reaction]>

Store at -20 °C

Components:

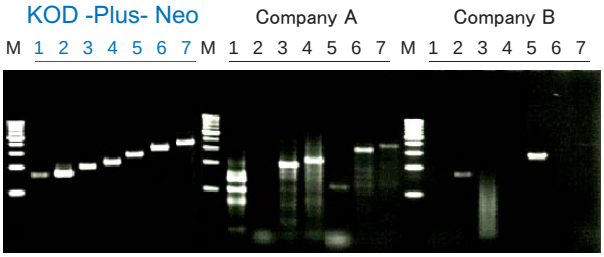
KOD -Plus- Neo (1.0 U/μl)*	200 μl
10 x PCR Buffer for KOD -Plus- Neo	1 ml
25 mM $MgSO_4$	1 ml
2 mM dNTPs	1 ml

*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize polymerase and 3'→5' exonuclease activity for hot start technology.



Features

- **Exhibits approximately 80-fold greater PCR fidelity than Taq DNA polymerase.**
- **"Elongation enhancer" enables greater amplification efficiency and elongation capability (up to 24 kb from human genomic DNA) compared to conventional PCR.**
- **Requires only 30 sec/kb for the PCR extension step.**
- **2-step cycle conditions can be used for amplification using ≥ 20 mer primers (melting temperatures, Tm >63°C)**
- **TArget Clone™ -Plus- [p29] can be applied to the TA cloning of blunt-end PCR products amplified using KOD -Plus- Neo.**



M: 1kb DNA Ladder
1: Human brk Tyrosine kinase (1.6kb)
2: Human rac protein kinase-alpha (1.7kb)
3: Human 63kDa protein kinase (2.0kb)
4: Human c-syn protooncogene (2.2kb)
5: Human FER Tyrosine kinase (2.7kb)
6: Human cell adhesion kinase beta (3.2kb)
7: Human Jak2 kinase (3.6kb)

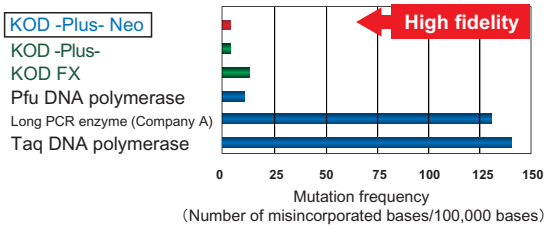


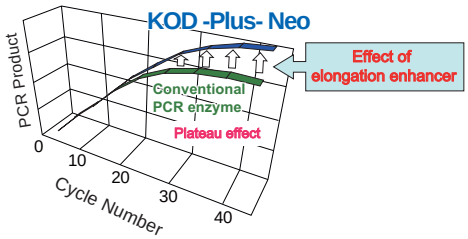
Fig. Amplification of various protein kinase targets

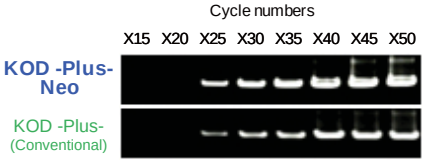
Applications

- High fidelity PCR
- Fast and efficient PCR

Principle

KOD -Plus-Neo is based on KOD DNA polymerase and is an improved version of KOD -Plus-. This enzyme contains a unique “elongation enhancer” that suppresses the “plateau effect” caused by conventional PCR. Therefore, KOD -Plus- Neo exhibits greater amplification efficiency and elongation capability compared to the previous version. Moreover, this enzyme requires only 30 sec/kb for the extension step during PCR. This facilitates high fidelity long range PCR.

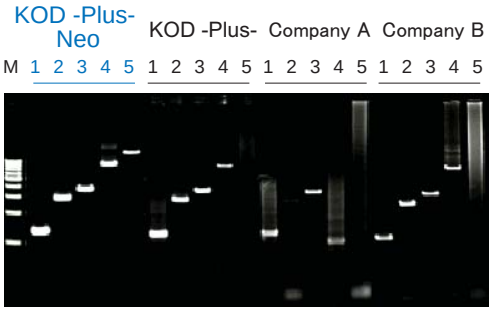




Application data

1. Amplification of long targets

Targets of various sizes were amplified from human genomic DNA by several PCR enzymes according to the recommended conditions of each enzyme. KOD -Plus- Neo successfully amplified targets up to 17.5 kb.



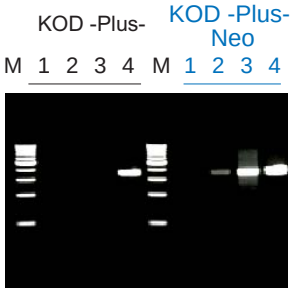
1: Human β-globin 1.3kb
2: Human β-globin 2.7kb
3: Human β-globin 3.6kb
4: Human β-globin 8.5kb
5: Human β-globin 17.5kb
M: 1kb DNA Ladder

[Cycling conditions of KOD −Plus− Neo]
1 ～ 4:
94°C, 2min.
98°C, 10sec.
68°C, 30sec./kb
30 cycles

5: Step-down cycle

2. Evaluation of the elongation rate

The β-globin gene (3.6 kb) was amplified from human genomic DNA (50 ng) using various extension times. KOD -Plus- Neo can amplify a 3.6 kb target using an extension time of 30 sec/kb.



Extension time : X
1: 36 sec. (10 sec./ kb)
2: 54 sec. (15 sec./ kb)
3: 108 sec. (30 sec./ kb)
4: 216 sec. (60 sec./ kb)

94°C, 2min.
98°C, 10sec.
68°C, X sec./kb
30 cycles

M: 1 kb DNA Ladder

References

1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.,* 63: 4504-10 (1997)
2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.,* 306: 469-77 (2001)
3) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem.,* 126: 762-8 (1999)

High success-rate PCR enzyme

KOD FX

KOD FX is based on DNA polymerase from the hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1(1)(2). KOD FX results in much greater PCR success based on efficiency and elongation capabilities than KOD -Plus- or other Taq-based PCR enzymes. KOD FX enzyme solution contains two types of anti-KOD DNA polymerase antibodies that inhibit polymerase and 3'→5' exonuclease activities, thus allowing for Hot Start PCR(3). KOD FX generates blunt-end PCR products, due to 3'→5' exonuclease (proof-reading) activity.

Code No. KFX-101 200 U
<200 reactions [50 μl per reaction]>

Store at -20 °C

Components:

KOD FX (1.0 U/μl)*	200 μl
2 x PCR Buffer for KOD FX	3 x 1.7 ml
2 mM dNTPs	2 x 1 ml

*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize polymerase and 3'→5' exonuclease activity for hot start technology.

Features

- **Enable the effective amplification from crude samples.**
- **Various microorganisms can be directly used as templates for PCR.**
- **Enable the effective amplification of difficult targets, such as high percentage GC or AT content, and/or long targets.**
- **Enables the following amplifications (Maximum):**
  40kb from phage lambda DNA, 24kb from human genomic DNA, and 13.5kb from cDNA.
- **The PCR error ratio is about 10 times less than that of Taq DNA polymerase.**
- **TArget Clone™ -Plus- [p29] can be applied to the TA cloning of blunt-end PCR products amplified using KOD FX.**

Applications

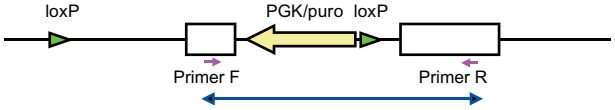
- Genotyping (Amplification from crude samples)
- Efficient amplification of difficult targets (high G/C, long) from genomic DNA or cDNA with fast mode
- High fidelity PCR

Application data

1. Comparison of PCR efficiency on mouse genotyping

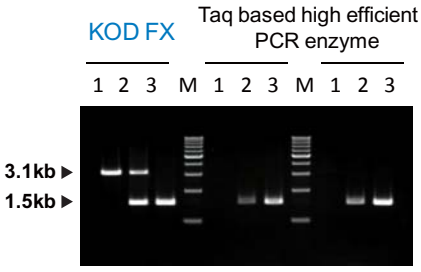
The target loci of the transgenic mice were amplified from the mouse tail lysates [see p6 Alkaline lysis methods] using KOD FX and the other Taq-based high efficient PCR enzymes. The target loci (3.1 kb) were successfully amplified by KOD FX.

Sample: Supernatant of mouse tail lysates 1 μl / 50 μl Reaction
Targeted locus (Tg)



[Cycling conditions of KOD FX]

94°C, 2min.
98°C, 10sec.
68°C, 2 min. } 30 cycles



M: 1kb DNA Ladder
1: Tg / Tg
2: Tg / W
3: W / W

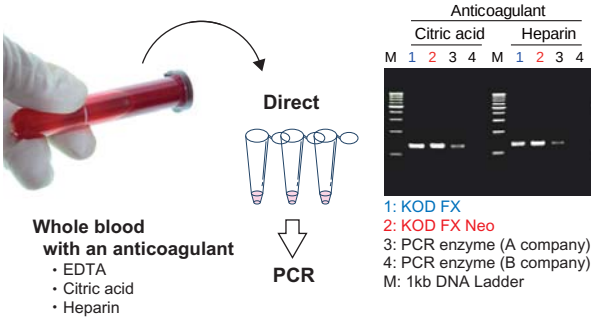
References

1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.,* 63: 4504-10 (1997)
2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.,* 306: 469-77 (2001)
3) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem.,* 126: 762-8 (1999)

Protocols for amplification from crude samples using KOD FX series

KOD FX series (KOD FX [p6] and KOD FX Neo [p8]) is suitable for amplification from crude specimens such as whole blood, various lysates (e.g. mouse tail, plant samples) and organisms bearing cell walls (*e.g.* yeast, fungi, gram-positive bacteria). We recommend the following protocols for efficient amplification.

Direct amplification from whole blood



DW	X (µl)
2x PCR Buffer	25
2 mM dNTPs	10
10 pmol/µl Primer F	1.5
10 pmol/µl Primer R	1.5
KOD FX or KOD FX Neo (1U/ml)	1
Whole blood / Lysates etc.	1~2*
Total	50

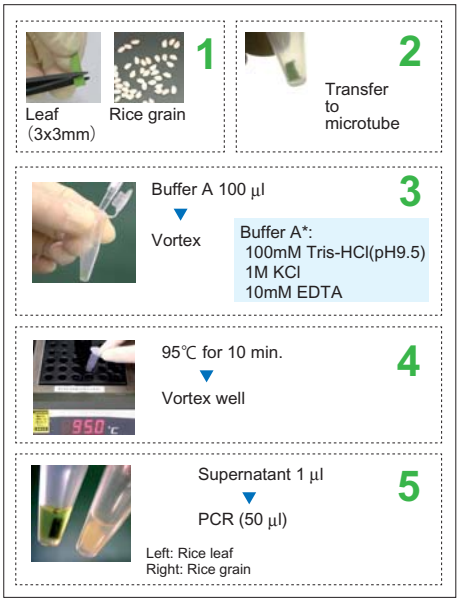
*In the case of microorganisms colony, the sample volume should be omitted.

Amplification from mouse tail lysates

Alkaline lysis method

Amplification from plant samples

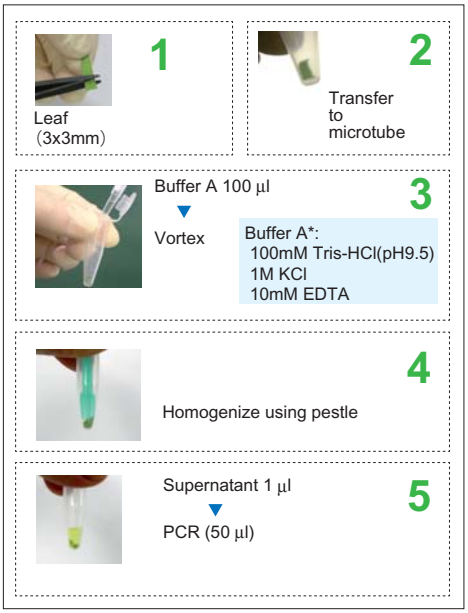
One step method



*BioTechniques, 19: 394 (1995)

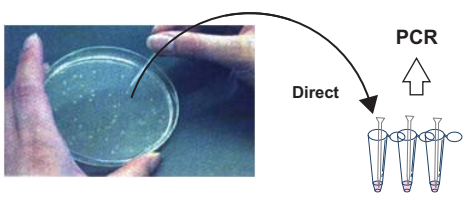
Homogenization method

This protocol is effective for the amplification of the targets on genomic DNA



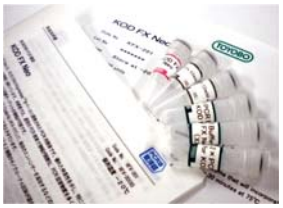
**BioTechniques*, 19: 394 (1995)

Direct amplification from yeast & fungus colonies



High efficient & high success-rate PCR enzyme

KOD FX Neo



Code No. KFX-201 200 U
<200 reactions [50 µl per reaction]>

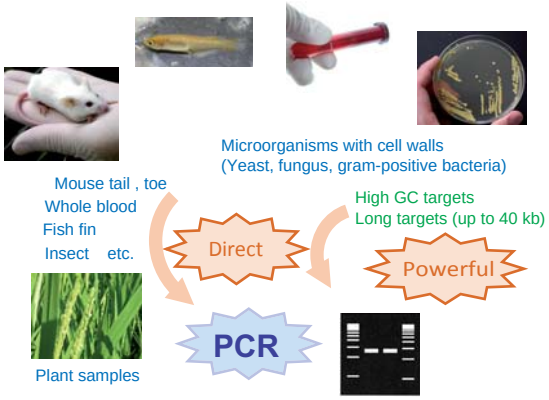
Store at -20 °C

Components:

KOD FX Neo (1.0 U/µl)*	200 µl
2 x PCR Buffer for KOD FX Neo	3 x 1.7 ml
2 mM dNTPs	2 x 1 ml

*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize polymerase and 3'→5' exonuclease activity for hot start technology.

KOD FX Neo is based on the DNA polymerase from the hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1(1)(2). This enzyme contains a unique "elongation enhancer" that suppresses the "plateau effect", enabling greater elongation rates and capabilities. Therefore, this enzyme provides greater efficiency and elongation capabilities on difficult targets (high GC content and long targets) than conventional PCR enzymes. In particular, this enzyme shows greater PCR success when using crude samples as templates.
The enzyme solution contains two types of anti-KOD DNA polymerase antibodies that inhibit the polymerase and 3'→5' exonuclease activities, thus allowing for hot start PCR(3).
This enzyme generates blunt-end PCR products because of its 3'→5' exonuclease (proof-reading) activity.
KOD FX Neo is the improved version of the previous KOD FX (Code No. KFX-101).



Features

- **Enable the effective amplification from crude samples.**
- **Various microorganisms can be directly used as templates for PCR.**
- **Enable the effective amplification of difficult targets, such as high percentage GC or AT content, and/or long targets.**
- **The PCR error ratio is about 10 times less than that of Taq DNA polymerase.**
- **"Elongation enhancer [p4]" enables greater amplification efficiency and elongation capability (up to 40 kb).**
- **Requires only 30 sec/kb for the PCR extension step (except for amplification from crude samples).**
- **TArget Clone™ -Plus- [p29] can be applied to the TA cloning of blunt-end PCR products amplified using KOD FX Neo.**

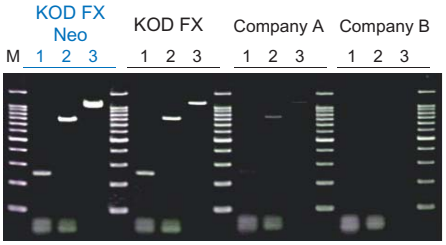
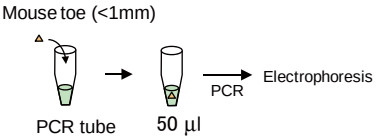


Fig. Direct amplification using mouse toes



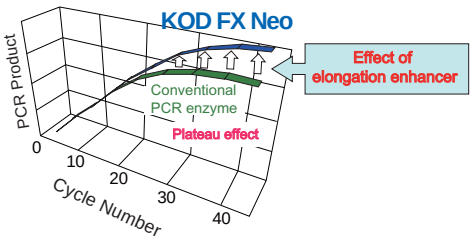
M : 200 bp DNA Ladder
1 : Mouse TATA box binding protein (TBP) 0.5 kb
2 : Mouse transferrin receptor (Tfr) 1.5 kb
3 : Mouse membrane glycoprotein (Thy-1) 2.6 kb

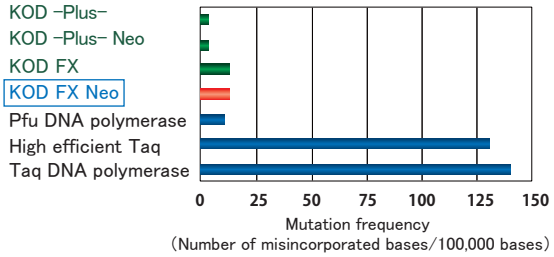
Applications

- Genotyping (Amplification from crude samples)
- Efficient amplification of difficult targets (high GC or AT content, long) from genomic DNA or cDNA with fast mode
- High fidelity PCR

Principle

KOD DNA polymerase exhibits excellent elongation efficiency and robustness for crude samples. KOD FX Neo is based on KOD DNA polymerase and is an improved version of KOD FX.
The KOD FX series results in much improved PCR success compared to conventional PCR enzymes. In particular, KOD FX Neo effectively amplifies difficult targets (*e.g.* high GC or AT content, long [up to 40 kb from human genomic DNA], etc.) due to the presence of the “elongation enhancer”.

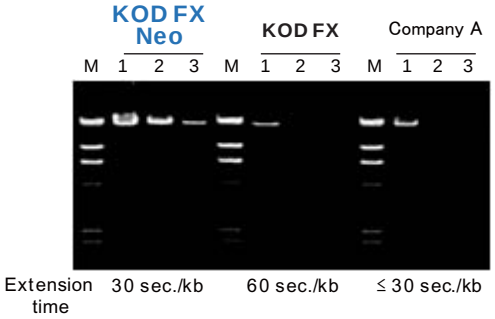




Application data

1. Amplification of long targets

Targets of various sizes were amplified from human genomic DNA by several PCR enzymes according to the recommended conditions of each enzyme. KOD FX Neo successfully amplified targets up to 40 kb.



M : λ/Hind III Marker
1: Human tPA 24kb
2: Human β-globin 32kb
3: Human β-globin 40kb

Template:
Human genomic DNA 200ng/50µl

KOD FX Neo

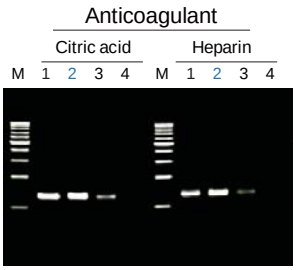
94℃	2min.	
98℃	10sec.	5 cycles
74℃	30sec./kb	
98℃	10sec.	5 cycles
72℃	30sec./kb	
98℃	10sec.	5 cycles
70℃	30sec./kb	
98℃	10sec.	20 cycles
68℃	30sec./kb	
68℃	7min.	
4℃	Hold	

KOD FX

94℃	2min.	
98℃	10sec.	5 cycles
74℃	60sec./kb	
98℃	10sec.	5 cycles
72℃	60sec./kb	
98℃	10sec.	5 cycles
70℃	60sec./kb	
98℃	10sec.	20 cycles
68℃	60sec./kb	
68℃	7min.	
4℃	Hold	

2. Amplification from blood specimens

Human β-globin gene (1.3 kb) was amplified using various PCR enzymes with whole blood specimens containing citric acid and heparin as anticoagulants. KOD FX Neo amplified the targets efficiently from both whole blood samples.



94°C	2min.	
98°C	10sec.	30 cycles
68°C	1.5min.	
4°C	Hold	

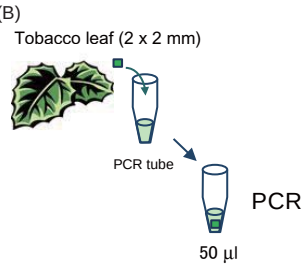
M : 1 kb DNA Ladder
1 : KOD FX
2 : KOD FX Neo
3 : Company A
4 : Company B

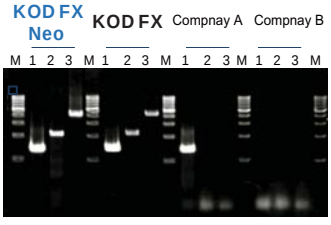
Template : Whole blood 5 µl
Target : Human β-globin 1.3 kb

3. Amplification from leaf samples

(A) Two targets (2.2 and 4.6 kb) were amplified using lysates from tobacco leaves. Each PCR reaction was performed according to the recommended conditions with 35 cycles. KOD FX Neo showed greater amplification from lysates prepared by the “one-step method”.
(B) Various targets were then directly amplified using small pieces of tobacco leaves (2 × 2 mm). KOD FX Neo successfully amplified DNA using these templates.

M : 1 kb DNA Ladder
1 : KOD FX
2 : KOD FX Neo





94°C	2min.	
98°C	10sec.	35 cycles
68°C	1min./kb	
4°C	Hold	

M : 1 kb DNA Ladder
1 : rbcL 1.3 kb
2 : rbcmT 2.2 kb
3 : rbcmT 4.6 kb

References

1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.,* 63: 4504-10 (1997)
2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.,* 306: 469-77 (2001)
3) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem.,* 126: 762-8 (1999)

KOD Dash

KOD Dash is a highly efficient DNA polymerase whose development was based on the Barns' method (1). This method uses a DNA polymerase that lacks a 3'→5' exonuclease (proofreading) activity and a small amount of an archaeal DNA polymerase with proofreading activity. In the reagent, the 3'→5' exonuclease activity-deficient mutant of KOD DNA polymerase <KOD exo(-)> and WT-KOD DNA polymerase are used. Because the proofreading activity repairs misincorporated nucleotide bases, which cause the termination of polymerase reactions, PCR with this mixed enzyme solution enables highly efficient amplification. KOD Dash generates blunt and dA overhang-ended PCR products.

Code No. LDP-101 250 U
<200 reactions [50 μl per reaction]>

Store at -20 °C

Components:

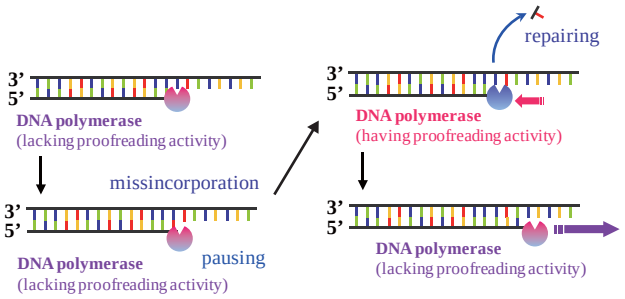
KOD Dash (2.5 U/μl)	100 μl
10 x PCR Buffer for KOD Dash*	1.2 ml
2 mM dNTPs	1.0 ml

*The final Mg concentration in the reaction mixture is 1.2 mM.

Features

- **Effective for the amplification of various targets from a small amount of starting template.**
- **Shows greater elongation velocity than Taq DNA polymerase (2 folds) and Pfu DNA polymerase (6 folds) due to the intrinsic property of KOD DNA polymerase.**
- **The PCR error rate of this enzyme mixture is approximately 3 times less than that of Taq DNA polymerase.**

Principle



Applications

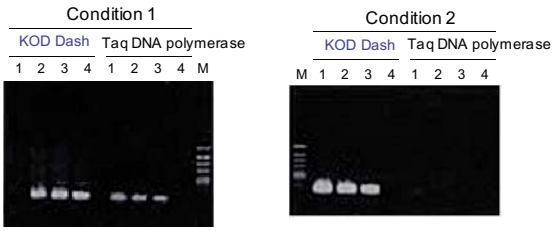
- Fast & Efficient PCR

Application data

1. Comparison of PCR efficiency on mouse genotyping

The sensitivities of PCR of KOD Dash and Taq DNA polymerase were compared using a normal cycle and fast cycle. HCV RNA from serum specimens was detected using the nested RT-PCR method reported by Okamoto et al. [*Japan. J. Exp. Med.* 60: 215-222 (1990)]. KOD Dash can rapidly detect low copies of HCV in serum specimens.

Template: HCV cDNA synthesized
from HCV RNA isolated from serum specimens
Target: HCV pre-core gene



[Cycling conditions]

Condition 1:
<1st cycle>
94°C, 20sec.
53°C, 30sec.
72°C, 1min.
38 cycles
<2nd cycle>
94°C, 20sec.
50°C, 30sec.
72°C, 1min.
28 cycles

Condition 2 (fast cycle):
<1st cycle>
98°C, 10sec.
55°C, 2sec.
74°C, 2sec.
38 cycles
<2nd cycle>
98°C, 10sec.
52°C, 2sec.
74°C, 2sec.
28 cycles

References

1) TW.M. Barns, *Proc. Natl. Acad. Sci.* USA, 91: 2216-2220 (1994)

Blend Taq® & Blend Taq® -Plus-

Code No. BTQ-101 250 U
(Blend Taq®)

BTQ-201 250 U
(Blend Taq® -Plus-: Hot start version)

<200 reactions [50 µl per reaction]>

Store at -20 °C

Components:

<Blend Taq®>	
Blend Taq® (2.5 U/µl)	100 µl
10x Buffer	1 ml
2 mM dNTPs	1 ml
<Blend Taq® -Plus->	
Blend Taq® -Plus- (2.5 U/µl)*	100 µl
10x Buffer	1 ml
2 mM dNTPs	1 ml

*Blend Taq® -Plus- contains anti-Taq DNA polymerase antibodies for the hot start technology.

Blend Taq® and Blend Taq® -Plus- are highly efficient Taq-based DNA polymerases whose development was based on the Barns' method (1)[p10]. This method uses a DNA polymerase that lacks 3'→5' exonuclease (proofreading) activity (*e.g.* Taq DNA polymerase) and a small amount of an archaeal DNA polymerase with proofreading activity. Because the proofreading activity repairs misincorporated nucleotide bases causing the termination of the polymerase reaction, PCR with a 'mixed' enzyme solution enables highly efficient amplification.
Blend Taq® -Plus- contains anti-Taq DNA polymerase antibodies that inhibit polymerase activity, allowing for hot start PCR.
Blend Taq® and Blend Taq® -Plus- generate blunt and dA overhang-ended PCR products. Therefore, the PCR products can be cloned using a standard TA-cloning method.

Features

- **Enables effective amplification of various targets from a small amount of starting template.**
- **The hot start technology enables highly efficient amplification. <Blend Taq® -Plus->**
- **The PCR error rate of this enzyme is approximately 3 times less than that of Taq DNA polymerase.**

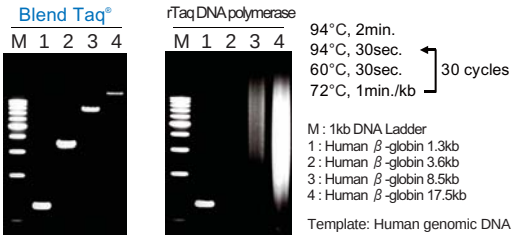


Fig. Comparison of the amplification efficiency

Fig. Comparison of the sensitivity

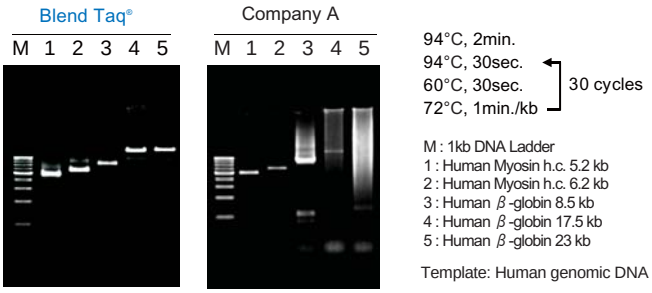
Applications

- Long range PCR
- Colony direct PCR

Application data

1. Amplification of long targets

The PCR performances of Blend Taq® and Blend Taq® -Plus- were evaluated by amplification of long targets. Blend Taq® and Blend Taq® -Plus- showed distinct bands whereas PCR enzymes from other companies showed poor bands or smears. No non-specific band was detected by Blend Taq® -Plus-.



References

1) W.M. Barns, *Proc. Natl. Acad. Sci.* USA, 91: 2216-2220 (1994)

Taq DNA Polymerase Master Mix (Hot Start)

Quick Taq® HS DyeMix

Code No. DTM-101 100 reactions
[50 µl per reaction]

Store at -20 °C

Components:

2x Quick Taq® HS DyeMix	2 x 1.25ml

*Quick Taq® HS DyeMix contains anti-Taq DNA polymerase antibodies for the hot start technology.

Quick Taq® HS DyeMix is a Taq-based 2X master mix PCR reagent that contains an electrophoresis dye (BPB; bromophenol blue) and anti-Taq antibodies for hot start PCR. This reagent contains all components for PCR except primers and template DNA. This reagent shows specific and efficient amplification. The amplified products can be directly loaded in the wells of an agarose or acrylamide gel.

Features

- **Quick Taq® HS Dye Mix contains bromophenol blue (BPB) as an electrophoresis dye; the PCR products can be analyzed directly on an agarose or acrylamide gel.**
- **Enables greater PCR performance than conventional Taq DNA polymerase.**
- **The hot start technology enables efficient and specific PCR.**
- **The master mix is stable for at least three months at 4°C. No decrease in reaction efficiency is observed following 30 freeze-thaw cycles.**

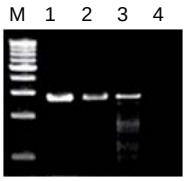
Applications

- General PCR
- Colony direct PCR

Application data

1. Amplification of the human p53 gene (2.9 kb)

The human p53 gene (2.9 kb) was amplified using 50 ng of human genomic DNA.Quick Taq® HS DyeMix successfully amplified the targets.



M : 1kb Ladder
1: Quick Taq® HS DyeMix
2: rTaq DNA polymerase (hot start)
3: rTaq DNA polymerase
4: Taq Master Mix (A company)

94°C, 2min.
94°C, 30sec.
60°C, 30sec.
68°C, 3min.
40 cycles

Template: Human genomic DNA 50 ng/ 50 µl reaction
Forward Primer : AATGGATGATTTGATGCTGTCCC
Reverse Primer : ATAAGAGCTCCCAAGACTTAG
*Final concentration 0.2 µM

2. Insert amplification by a colony-direct PCR

The inserts were amplified using Quick Taq® HS DyeMix with universal primers from *E. coli* DH5α colonies bearing pTA2 plasmid (insert size: 500 bp). Quick Taq® HS DyeMix successfully and efficiently amplified all targets.

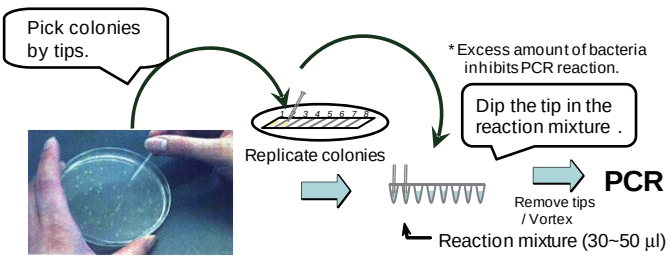
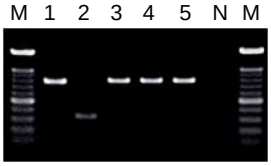


Fig. Flow chart of colony-direct PCR using *E. coli* cells



94°C, 2min.
94°C, 30sec.
60°C, 30sec.
68°C, 1min.
25 cycles

M: 100 bp Ladder Markers
1: Colony (insert +)
2: Colony (insert -)
3: Colony (insert +)
4: Colony (insert +)
5: Colony (insert +)
N: Negative control
M: 100 bp Ladder Markers

Forward Primer : CGCCAGGGTTTTCCCAGTCACGAC
Reverse Primer : AGCGGATAACAATTTCACACAGGAAAC
*Final concentration 0.2 µM

Standard PCR enzyme

rTaq DNA Polymerase

Taq DNA polymerase is the most widely used thermostable DNA polymerase derived from the thermophilic bacteria *Thermus aquaticus* (Taq) YT-1. The enzyme possesses a 5'→3' polymerase activity and a double-strand specific 5'→ 3' exonuclease activity.

Code No. TAP-201 250 U

<100~200 reactions [50 µl per reaction]>

Store at -20 °C

Components:

rTaq DNA Polymerase (5 U/µl)	50 µl
10× Buffer*	1 ml
25 mM $MgCl_2$	1 ml
2 mM dNTPs	1 ml

* 100 mM Tris-HCl (pH 8.3), 500 mM KCl

Features

- **Tolerates various kinds of PCR protocols.**
- **Applicable for hot start technology by adding anti-Taq antibody "anti-Taq high " (Code No. TCP-101) [p27].**
- **PCR products can be cloned by using a TA cloning method.**
- **Incorporates dUTP, dITP, and fluorescently-labeled nucleotides.**

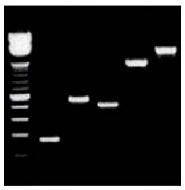
Applications

- PCR
- Primer extension

References

1) F.C. Lawyer, S. Stoffel, R.K. Saiki, K. Myambo, R. Drummond, D.H. Gelfand., *J. Biol. Chem*., 264: 6427-6437 (1989)
2) T. Nagahama, K. Sugiura, S. Lee, H. Morita, Y. Adachi, A.H. Kwon, Y. Kamiyama, S. Ikehara, *Stem cells*, 19: 425-435 (2001)

Application data



M: 100bp Ladder
1: 180bp p53 exon8
2: 444bp p53 exon8
3: 408bp β-globin
4: 1kb β-globin
5: 1.3kb β-globin

DW	X (µl)
10x PCR Buffer	5
2 mM dNTPs	5
25 mM $MgCl_2$	3
10 pmol/µl Primer F	1
10 pmol/µl Primer R	1
rTaq DNA polymerase (5 U/µl)	[1.25~2.5 U]
Template DNA	Y
Total	50

94°C, 2min.
94°C, 10sec.
(Tm-5)°C, 30sec.
68°C, 1min./ kb
25~40 cycles

Multi-functional PCR enzyme

rTth DNA Polymerase

Tth DNA polymerase is a thermostable DNA polymerase derived from the thermophilic bacteria *Thermus thermophilus* (Tth) HB8.
The enzyme exhibits reverse transcriptase activity in addition to 5'→3' polymerase activity and double strand specific 5'→ 3' exonuclease activity in the presence of Mn^{2+} ions: therefore, enabling one-step RT-PCR. Kits for one-step RT-PCR (Code No. PCR-311, p14) and real-time PCR (Code No. QRT-101, 201, p20) using this enzyme are available.

Code No. TTH-301 250 U

<100~200 reactions [50 µl per reaction]>

Store at -20 °C

Components:

rTth DNA Polymerase (5 U/µl)	50 µl
10× Buffer (contains $MgCl_2$)*	1 ml
Dilution buffer	1 ml
2 mM dNTPs	1 ml

* This buffer is optimized for PCR, not one-step RT-PCR.

References

1)T.W. Myers, D.H. Gelfand, *Biochemistry*, 30: 7661-7666 (1991).
2) K. Yamada, M. Terashima, M. Shimoyama, M. Tsuchiya, *J Biochem*. 130: 335-40 (2001)

RT-PCR Quick Master Mix

Code No. PCR-311 50 reactions
[50 µl per reaction]

Store at -20 °C

Components:

This reagent includes the following components for 50 reactions, 50 µl total reaction volume.

2 x RT-PCR Quick Master Mix	2 x 625 µl
50 mM $Mn(OAc)_2$	200 µl
Nuclease-free water	1100 µl
Positive control RNA (human G3PDH mRNA, 5 x 10^5copies/µl)	50 µl
Control Primer F (10 pmol/µl)	50 µl
Control Primer R (10 pmol/µl)	50 µl

RT-PCR Quick Master Mix provides a 2x master mix for RT-PCR using a thermostable DNA polymerase derived from *Thermus thermophilus* (Tth) HB8 (1). Tth DNA polymerase exhibits reverse transcriptase activity in addition to DNA polymerase activity in the presence of Mn^{2+} ions. Therefore, this system enables "one-step RT-PCR" including reverse transcription and PCR steps. This kit is suitable for high-throughput RT-PCR, and decreases contamination risks.

Features

- **Enables one-step RT-PCR including reverse transcription and PCR steps.**
- **Tth DNA polymerase can work at a higher temperature. Therefore, this kit has the advantage of amplifying targets with complex conformations and GC-rich content.**
- **The hot start technology enables highly efficient and specific amplification.**

Applications

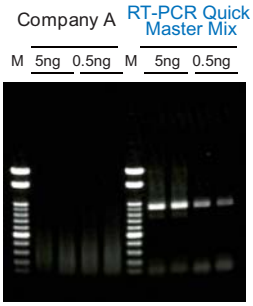
- One-step RT-PCR

Application data

1. Detection of cdc2 mRNA using one-step RT-PCR

The human cdc2 gene (900 bp) was amplified using mRNA (5 ng and 0.5 ng) purified from a human cell line. Target genes were successfully amplified using the RT-PCR Quick Master Mix.

Primer:
Cdc2-F : 5'-CCATACCATTGACTAACTATGGAAGAT-3' (27mer)
Cdc2-R : 5'-GTCAGAAAGCTACATCTTCTTAATCTG-3' (27mer)



90°C, 30 sec.
60°C, 30 min. <Reverse transcription>
94°C, 1 min.
94°C, 30 sec.
60°C, 30 sec.] 40 cycles
72°C, 1 min.
72°C, 7 min.

Sample : human mRNA
5ng or 0.5ng/ 50 µl reaction mixture
Target : cdc2 mRNA ORF 900bp
M: 100bp DNA Ladder

Fig. Amplification of human cdc gene (900 bp).

References

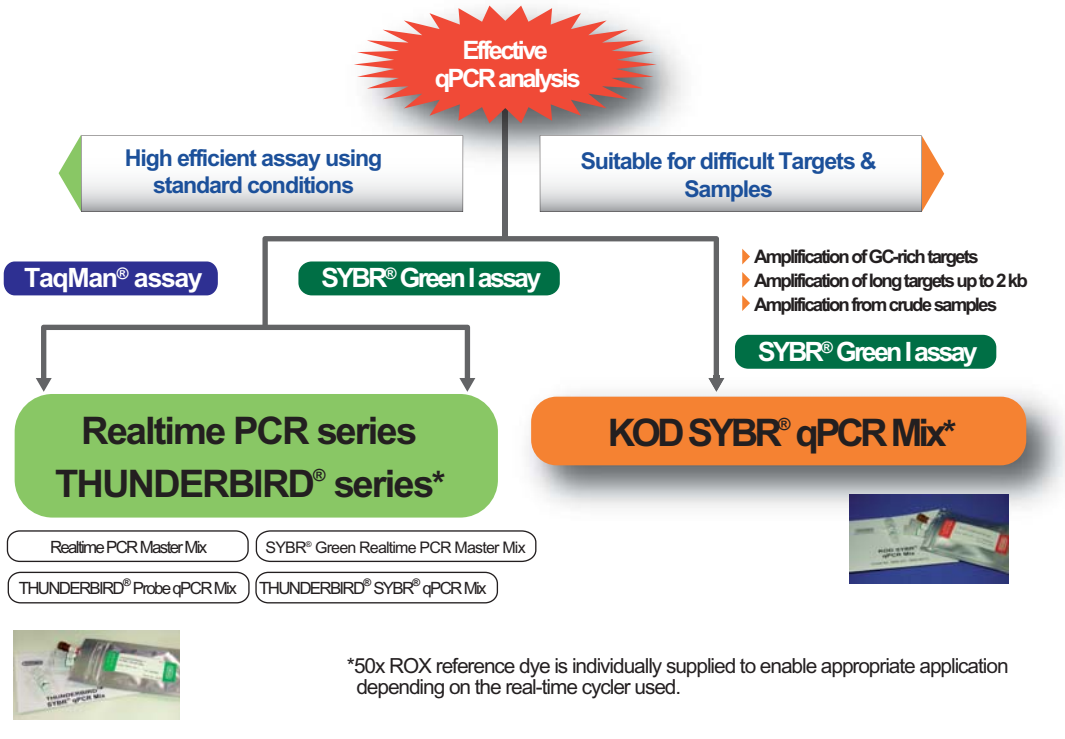
1) Myers T. W. and Gelfand D. H. , *Biochemistry*, 30: 7661-6 (1991)

Real-time PCR Master Mix selection guide

Toyobo has various real-time PCR master mixes to choose from. The following table shows the characteristics of each product. The realtime PCR Master Mix series and the THUNDERBIRD® series are Taq DNA polymerase-based 2 × master mixes for real-time PCR, which contain all of the necessary reaction components, except for the primer and probe. The THUNDERBIRD® series shows reduced primer-dimer formation and Rox dye is provided separately to be enable appropriate application depending on the real-time cycler used. The *RNA-direct*™ series is a 2 × master mix for one-step real-time PCR using a thermostable DNA polymerase derived from *Thermus thermophilus* (Tth) HB8. Tth DNA polymerase exhibits reverse transcriptase activity in the presence of Mn^{2+} ions. This system allows for one-step real-time PCR, including reverse transcription and PCR steps. KOD SYBR® qPCR Mix contains a 3'→5' exonuclease deficient KOD DNA Polymerase and an optimized buffer. The reagent can also be used for the amplification, of long target (<2 kb) and GC rich targets. And the reagent is also applicable to the amplification from crude samples such as whole blood, microorganisms and various lysates from animal and plant tissues.

Enzyme	Product Name	Assay type	One-step RT-PCR	Hot start (Antibody)	Passive referrence	Glass capillary	Specificity	Efficiency	Long target amplification	GC-rich targets	Amplification from crude samples	Reference page
Taq DNA polymerase	Realtime PCR Master Mix	Probe		✓	✓	✓	+++	++	+	+	+	16
	SYBR® Green Realtime PCR Master Mix	SYBR		✓	✓	✓	+	++	+	+	+	
	THUNDERBIRD® Probe qPCR Mix	Probe		✓	✓	✓	+++	++	+	+	+	16
	THUNDERBIRD® SYBR® qPCR Mix	SYBR		✓	✓	✓	++	++	+	+	+	
Tth DNA polymerase	*RNA-direct*™ Realtime PCR Master Mix	Probe	✓	✓	✓	✓	+++	++	+	++	++	20
	RNA-direct™ SYBR® Green Realtime PCR Master Mix	SYBR	✓	✓	✓	✓	++	++	+	++	++	
KOD exo(-) DNA polymerase	KOD SYBR® qPCR MixR Master Mix	SYBR		✓	✓	✓	++	++	+++ (<2kb)	+++	+++	18

+++: Best, ++: Excellent, +: Good, ✓: Applicable *50x ROX reference dye is individually supplied.



*50x ROX reference dye is individually supplied to enable appropriate application depending on the real-time cycler used.

High efficient real-time PCR Master Mix

Realtime PCR Master Mix Series

Code No. QPK-101 (TaqMan® probe version)
QPK-201 (SYBR® Green version)
<200 reactions [50 μl per reaction]>

Store at -20 °C

Components:

<QPK-101 TaqMan® probe version>	
Realtime PCR Master Mix	5 x 1 ml
<QPK-201 SYBR® Green version>	
SYBR® Green Realtime PCR Master Mix	5 x 1 ml

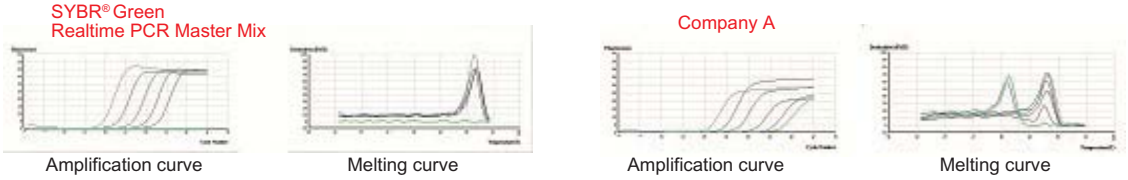
Realtime PCR Master Mix Series is a Taq DNA polymerase-based 2x master mix for real-time PCR, which contains all components, except for the primer and probe. Realtime PCR Master Mix is applicable in TaqMan® assays or hybridization probe assays, in combination with each probe. SYBR® Green Realtime PCR Master Mix is applicable for intercalation assay with SYBR® Green I.

Features

- **Applicable to a passive reference system (*e.g.*, ABI PRISM® 7700, Applied Biosystems, Inc.).**
- **Applicable to a glass capillary system (*e.g.*, LightCycler®, Roche Molecular Systems, Inc.).**

Application data

Amplification of the β-actin gene was detected using serially diluted genomic DNA solutions (10n dilution; 30 ng-3 mg) with real-time PCR kits for the SYBR® Green assay. SYBR® Green Realtime PCR Master Mix [Code No. QPK-201] showed greater sensitivity and efficiency than other kits (company A). Moreover, SYBR® Green Realtime PCR Master Mix generated fewer primer dimers than the other kits.



High efficient real-time PCR Master Mix

THUNDERBIRD® qPCR Mix Series

Code No. QPS-101 (TaqMan® probe version)
QPS-201 (SYBR® Green version)
200 reactions [50 μl per reaction]

Store at -20 °C

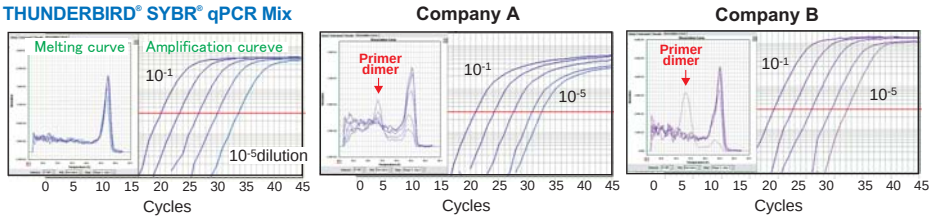
Components:

<QPS-101 TaqMan® probe version>	
THUNDERBIR® Probe qPCR Mix	3 x 1.67 ml
50x ROX reference dye	250 μl
<QPS-201 SYBR® Green version>	
THUNDERBIRD® SYBR® qPCR Mix	3 x 1.67 ml
50x ROX reference dye	250 μl

THUNDERBIRD® Probe and SYBR® qPCR Mix is a Taq DNA polymerase-based highly efficient 2x master mix for real-time PCR using TaqMan® probes and SYBR® Green I. The master mix contains all required components, except for ROX reference dye, probe and primers (50x ROX reference dye is individually supplied with this kit). The master mix facilitates reaction setup, and improves the reproducibility of experiments.
These products are improved versions of Realtime PCR Master Mix (Code No. QPK-101) and SYBR® Green Realtime PCR Master Mix (Code No. QPK-201) . In particular, reaction specificity and PCR efficiency is enhanced.

Features

- **The specificity for the detection of low-copy targets is improved.**
- **The dispersion of PCR efficiency between targets is reduced by a new PCR enhancer*. (*Patent pending)**
- **High specificity and effective amplification enable the detection of a broad dynamic range.**
- **Applicable to most real-time cyclers (*i.e.* block type and glass capillary type).**



β-actin mRNA was detected with serially diluted cDNA from HeLa cell total RNA. THUNDERBIRD® SYBR® qPCR Mix [Code No. QPS-201] showed greater sensitivity and efficiency than other kits (companies A and B).

Fig. Comparison of the sensitivity and efficiency of the SYBR® Green I assay

Applications

- Real-time PCR

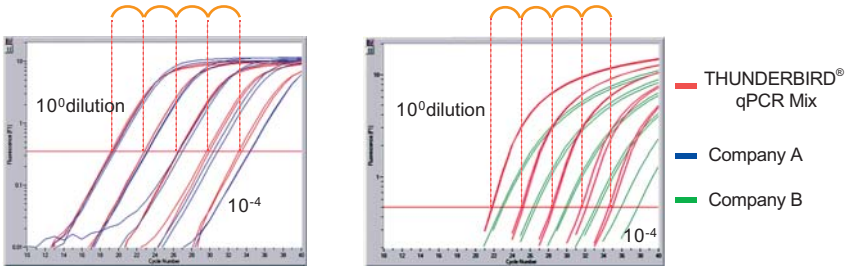
Table. Compatible real-time instruments

Applied Biosystems	ABI PRISM® 7000 ABI PRISM® 7700 Applied Biosystems® 7300 Applied Biosystems® 7500/7500FAST Applied Biosystems® 7900HT Applied Biosystems® StepOne™ Applied Biosystems® StepOnePlus™	**Roche Diagnositics**	LightCycler® 1.x / 2.0 LightCycler® Nano LightCycler® 480
		Bio-Rad/MJ	MiniOpticon™ CFX96 Touch™
		Agilent Technologies	Mx3000P/ Mx3005P/ Mx4000
		TaKaRa	Thermal Cycler Dice® Real Time System

Application data

1. Comparison of the PCR efficiency

Norovirus G1 and human GAPDH genes were detected using serially diluted cDNA samples by SYBR® Green I and TaqMan® real-time PCR. THUNDERBIRD® qPCR Master Mix showed greater efficiency than other reagents.

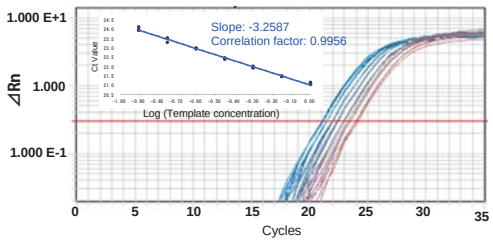


Detection of Norovirus G1 gene by SYBR® Green I assay. [Roche Diagnostics LightCycler 1.1]

Detection of human GAPDH gene by TaqMan® assay. [Roche Diagnostics LightCycler 1.1]

2. Verification of the measurement accuracy

Human GAPDH genes were detected using serially 20.5 fold diluted cDNA synthesized from HeLa cell total RNA by SYBR® Green I assay (n=4). THUNDERBIRD® SYBR® qPCR Mix successfully detected the differences between dilutions.



Detection of human GAPDH gene by SYBR® Green I assay. [Applied Biosystems 7900HT]

KOD SYBR® qPCR Mix

High GC Long Crude

Code No. QKD-201
200 reactions [50 μl per reaction]

Store at -20 °C
Components:

KOD SYBR® qPCR Mix	3 x 1.67 ml
50x ROX reference dye	250 μl

KOD SYBR® qPCR Mix is a highly efficient 2x master mix for real-time PCR using SYBR® Green I and the 3'→5' exonuclease deficient KOD DNA Polymerase. The master mix contains all of the required components, except for the primers and the ROX reference dye (50x ROX reference dye is supplied separately with this kit). The master mix decreases reaction setup time and improves the reproducibility of experiments.

Features

- **Quantitative amplification can be achieved using long targets, up to 2kb.**

<Electrophoresis analysis>

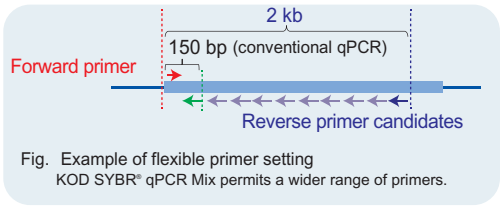


Fig. Example of flexible primer setting
KOD SYBR® qPCR Mix permits a wider range of primers.

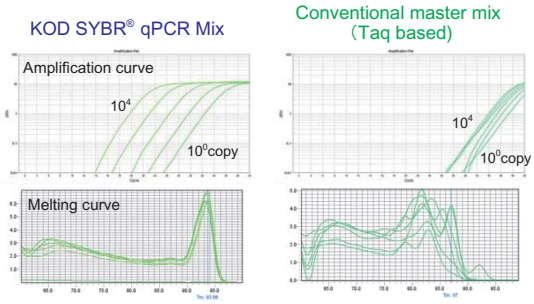
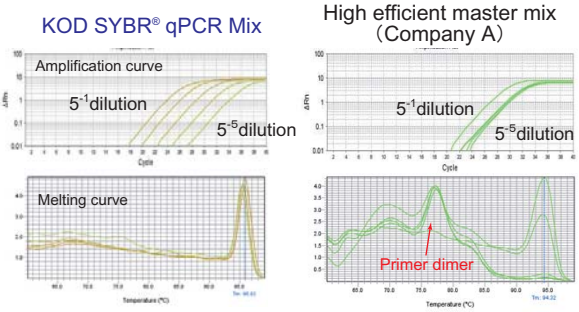


Fig. Long target amplification [ABI StepOnePlus™]
A real-time PCR assay was performed using primers for conventional PCR. KOD SYBR® qPCR Mix exhibited quantitative amplification.

- **Efficient for GC-rich targets**



Target: IGF2R (189 bp / GC content: 83%)
Template: HeLa cDNA was synthesized using ReverTra Ace® qPCR RT Kit (Code No.FSQ-101) with total RNA from HeLa cells.

Fig. Amplification of GC rich targets [ABI StepOnePlus™]

GC rich targets (GC content: >70%) were amplified using various real-time PCR master mixes. The targets were amplified successfully and quantitatively using KOD SYBR® qPCR Mix.

Applications

- Real-time PCR

Table Compatible real-time instruments

Applied Biosystems	ABI PRISM® 7000 ABI PRISM® 7700 Applied Biosystems® 7300 Applied Biosystems® 7500/7500Fast Applied Biosystems® 7900HT Applied Biosystems® StepOne™ Applied Biosystems® StepOnePlus™	**Roche Diagnositics**	LightCycler® 1.x / 2.0 LightCycler® Nano LightCycler® 480
		Bio-Rad/MJ	MiniOpticon™ CFX96 Touch™
		Agilent Technologies	Mx3000P/ Mx3005P/ Mx4000
		TaKaRa	Thermal Cycler Dice® Real Time System

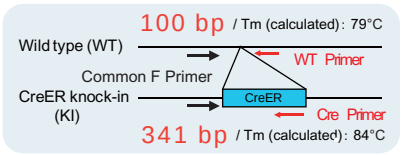
Enable the effective amplification from crude samples.

Effective amplification can be achieved using crude samples, as shown in the table. This reagent can be used for genotyping or SNP analysis using crude specimens.

Table Applicable samples

whole blood	ca. 1% (final)
nail (toe)	ca. 1mm
hair root	1~2 mm
oral mucosa	suspension
cultured cells	~ 10^3 cells
animal tissue	lysate (p 7)
plant tissue	lysate (p 7)

● Genotyping of knock-in mice usnig mouse-tail lysates



Target: Region contains the targeting site (Cre ER) (WT: 100 bp, KI: 341 bp)

Template: Mouse tail lysate (alkaline lysis method, p 7)
Primer ratio: F: WT: Cre = 0.2: 0.2: 0.67 mM (final)
Sample: Mouse tail lysate 2 µl / 20 µl reaction
Cycling condition:

98°C, 2 min
↓
98°C, 10 sec
60°C, 10 sec
68°C, 30 sec*
] 40 cycles
↓
Melting curve analysis

* Extension time should be set 30 sec/ 500 bp

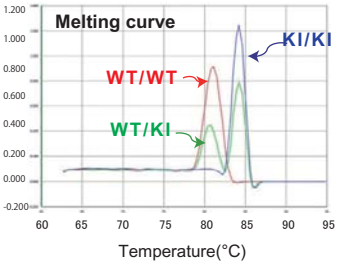
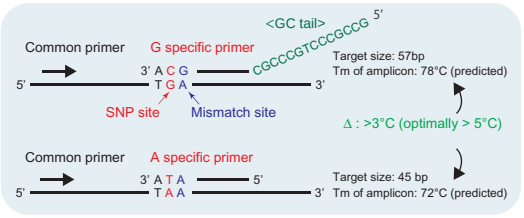
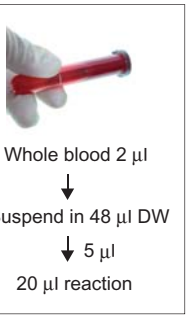


Fig. One-tube mouse genotyping using melting curve analysis [ABI 7500 Fast]

Primers were designed so that the amplicons were 100 bp (Tm: 79°C) and 341 bp (Tm: 84°C) for wild-type and knock-in, respectively. All genotypes were successfully detected.

● SNP analysis usnig whole blood samples





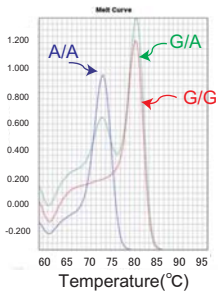


Fig. One-tube ASP-PCR analysis using whole blood specimen. [ABI 7500 Fast real-time PCR system]

SNP analysis was performed with a GC tailed primer from whole blood samples using KOD SYBR® qPCR Mix. All types of SNP were successfully determined by KOD SYBR® qPCR Mix. No signal was detected using the Taq-based conventional master mix (data not shown).

Principle

KOD SYBR® qPCR Mix was developed based on the unique properties (high efficiency, robustness) of KOD DNA Polymerase to enhance the convenience and versatility of the SYBR® Green I assay.

Table Comparison of properties with the conventional master

	Conventional (Taq based)	KOD SYBR® qPCR Mix
Enzyme	Taq DNA	KOD DNA Polymerase [exo(-) mutant]
Amplification size	70 ~ 150 bp (Maximum: 300 bp)	70 bp ~ 2 kb
High GC Targets	Susceptible	Not susceptible
Inhibition by impurities in crude samples	Susceptible	Not susceptible (Suitable for amplification from crude specimens)

Application data

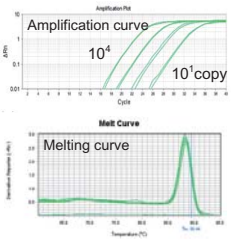
1. Comparison of the PCR efficiency

A promoter region having a typical CpG island was amplified using KOD SYBR® qPCR Mix and a conventional master mix with Taq DNA Polymerase. A quantitable detection was shown by KOD SYBR® qPCR Mix depending on the concentration of the template DNA. The conventional qPCR master mix based on Taq DNA Polymerase generated primer dimers mainly.

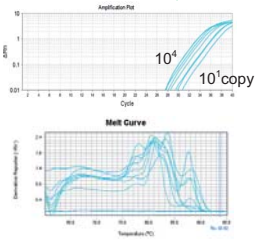
CCCGCCCGAGAGAGTGACTCTCACGAGAGCCGCGAGAGTCAGCT
TGGCCAATCCGTGCGGTCGGCGGCCGCTCCCTTTATAAGCCGACT
CGCCCGGCAGCGCACCGGGTTGCGGAGGGTGGGCCTGGGAGGG
GTGGTGGCCATTTTTTGTCTAACCCTAACTGAGAAGGGCGTAGGC
GCCGTGCTTTTGCTCCCCGCGC**GCTGTTTTTCTCGCTGACTT**

Target: GC content : 64%, 219 bp : Homo sapiens telomerase RNA (TR) gene, promoter and complete squence
Template: human genomic DNA
Primer: (from a paper using the ChIP technique) :
Blue: primer sequnce

KOD SYBR® qPCR Mix



Conventional master mix (Taq based)



One-step real-time RT-PCR Master Mix

RNA-direct™ Realtime PCR Master Mix Series

Code No. QRT-101 (TaqMan® probe version)
QRT-201 (SYBR® Green version)
100 reactions [50 μl per reaction]

Store at -20 °C

Components:

<QRT-101 TaqMan® probe version>

RNA-direct™ Realtime PCR Master Mix	5 x 0.5 ml
50 mM $Mn(OAc)_2$	0.5 ml

<QRT-201 SYBR® Green version>

RNA-direct™ SYBR® Green Realtime PCR Master Mix	5 x 0.5 ml
50mM $Mn(OAc)_2$	0.5 ml

RNA-direct™-direct™ Realtime PCR Master Mix is a 2x master mix for one-step real-time PCR using a thermostable DNA polymerase derived from *Thermus thermophilus* (Tth) HB8 [p13]. Tth DNA polymerase exhibits reverse transcriptase activity in the presence of Mn^{2+} ions. This system allows for one-step real-time PCR, including reverse transcription and PCR steps. *RNA-direct*™ Realtime PCR Master Mix is applicable for TaqMan® assay or hybridization probe assay, in combination with each probe. *RNA-direct*™ SYBR Green® Realtime PCR Master Mix can be applied to an intercalation assay with SYBR® Green I.

Features

- **Suitable for high-throughput real-time PCR and increases reliability of product, due to lowered risk of contamination.**
- **Applicable to a passive reference system (*e.g.*, ABI PRISM® 7700, Applied Biosystems, Inc.).**
- **Applicable to a glass capillary system (*e.g.*, LightCycler, Roche Molecular Systems, Inc.).**

Applications

- One-step real-time PCR

Application data

1. Correlation between one-step and two-step methods

Correlation between the one-step method (*RNA-direct*™ SYBR® Green Realtime PCR Master Mix [Code No. QRT-201]) and conventional 2-step method (ReverTra Ace® [Code No. TRT-101] (highly efficient reverse transcriptase)->SYBR® Green Realtime PCR Master Mix [Code No.QPK-201]) was evaluated based on the expression of Protein kinase δ (PKCδ) mRNA. The results from the one-step method were found to be highly correlated with those of the two-step method.

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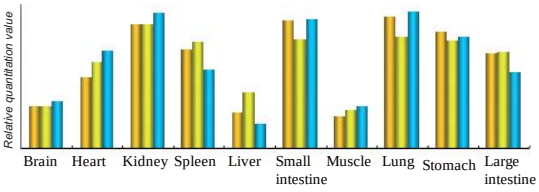


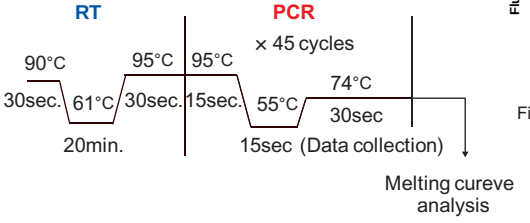
Fig. Comparison of the quantitation of target mRNA

The Y-axis indicates the relative quantitation value of Protein kinase (PKCδ) mRNA

Yellow bar (left): One-step method using 50 ng total RNA
Yellow bar (right): One-step method using 200 ng total RNA
Blue bar: Two-step method

2. Verification of the measurement accuracy

Amplification of G3PDH mRNA was detected using serially diluted poly (A)+ RNA (10^n dilution) with SYBR® Green Realtime PCR kits including Tth DNA polymerase. *RNA-direct*™ SYBR® Green Realtime PCR Master Mix [Code No. QRT-201)] showed greater sensitivity and signal intensity than the other kit (company A).



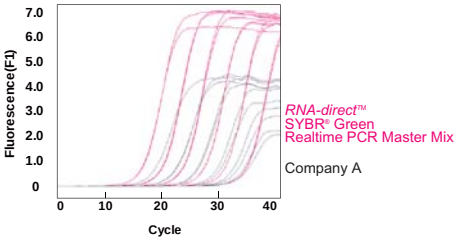


Fig. Comparison of the SYBR® Green assay

cDNA synthesis kits selection guide

Toyobo has various kits for cDNA synthesis based on the genetically improved M-MLV reverse transcriptase "ReverTra Ace®". ReverTra Ace -α-® is an efficient and convenient kit to synthesize high quality cDNA. This kit contains the highly efficient reverse transcriptase "ReverTra Ace®", as well as other components optimized for the synthesis of long cDNAs suitable for RT-PCR.
ReverTra Ace® qPCR RT Kit (Code No. FSQ-101) is an efficient and convenient kit to synthesize high quality cDNAs for real-time PCR. This kit contains the highly efficient reverse transcriptase "ReverTra Ace®" and a RT buffer optimized for the highly efficient synthesis of short-chain cDNAs suitable for real-time PCR. ReverTra Ace® qPCR Master Mix (Code No. FSQ-201) is a premix version of the ReverTra Ace® qPCR RT Kit.
ReverTra Ace® qPCR RT Master Mix with gDNA remover (Code No. FSQ-301) is an efficient and convenient kit, consisting of master mix reagents, to synthesize high quality cDNAs for real-time PCR. The kit includes reagents for reverse transcription and for the removal of genomic DNA. The protocol consists of i) a genomic DNA degradation step using "gDNA remover [DNase I]" and ii) a reverse transcription step. The two steps can be achieved sequentially without purification or heat inactivation of DNase I.

Enzyme	Product Name	Application	Efficiency	Long cDNA synthesis	Master Mix	Genomic DNA removal	Recommend PCR enzyme	Reference page
ReverTra Ace®	ReverTra Ace -α-®	Conventional RT-PCR	+	++ (> 10 kb)			KOD -Plus- series KOD FX series Taq-based PCR reagent	22
	ReverTra Ace® qPCR RT Kit	qPCR	++	+ (< 5 kb)			THUNDERBIRD® series Realtime PCR Master Mix series KOD SYBR® qPCR Mix KOD -Plus- series KOD FX series Taq-based PCR reagent	23
	ReverTra Ace® qPCR RT Master Mix	qPCR	++	+ (< 5 kb)	✓			
	ReverTra Ace® qPCR RT Master Mix with gDNA remover	qPCR	++	+ (< 5 kb)	✓	✓		24

++: Excellent, +: Good, ✓ : Applicable

High Efficient Reverse Transcriptase

ReverTra Ace®

ReverTra Ace® is a high efficient M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase that has been genetically modified to remove RNase H activity and increase reaction efficiency. It is the preferred enzyme for applications requiring full-length cDNAs and high product yields from total RNA, mRNA, rRNA, etc.

Code No. TRT-101 10,000 U

Store at -20 °C

Components:

ReverTra Ace® (100 U/µl)	100 µl
5 x Buffer	1 ml

Features

- **RNase minus M-MLV RTase with improved performance.**
- **Enables the synthesis of longer cDNAs (≥ 14 kb) than the wild-type enzyme.**
- **Exhibits excellent reaction efficiency at high temperatures.**

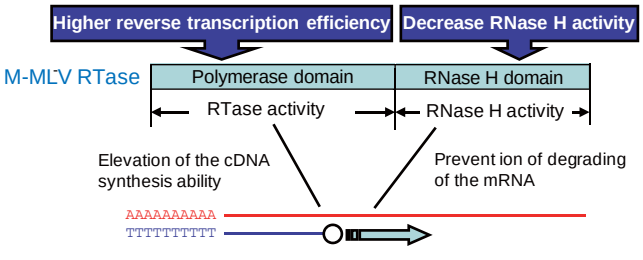


Fig. Genetically improved M-MLV Reverse transcriptase

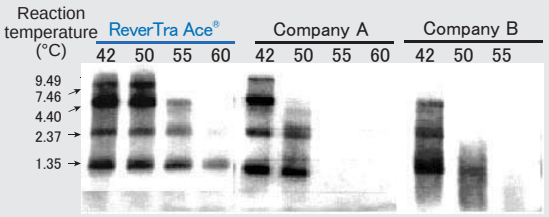


Fig. Comparison of elongation capability of RNase H minus RTases at various temperatures

Unit definition

One unit is defined as the amount of enzyme required to incorporate 1 nmole of dTTP into an acid-insoluble material in 10 min at 42ºC.

High efficient cDNA synthesis kit

ReverTra Ace -α-®

Code No. FSK-101 100 reactions
[20 μl per reaction]

Store at -20 °C

Components:

ReverTra Ace®	100 μl
5xRT buffer (contains 25 mM Mg^{2+})	400 μl
RNase inhibitor (10 U/μl)	100 μl
dNTPs mixture (10 mM)	200 μl
RNase-free H_2O	1200 μl
Oligo $(dT)_{20}$ (10 pmol/μl)	100 μl
Random primers (25 pmol/μl)	100 μl
Control Primer F (10 pmol/μl)	50 μl
Control Primer R (10 pmol/μl)	50 μl
Positive control RNA (10^5 copies/μl)	50 μl

ReverTra Ace -α-® is an efficient and convenient kit to synthesize high quality cDNA. This kit contains the highly efficient reverse transcriptase "ReverTra Ace®" , as well as other components optimized for the synthesis of long cDNAs suitable for RT-PCR. ReverTra Ace® is an M-MLV reverse transcriptase that has been improved by point mutation technology. ReverTra Ace® has two mutations in the polymerase and RNase H domains.

Features

- **All components for reverse transcription are included.**
- **ReverTra Ace® and the optimized buffer enable the synthesis of cDNAs ≥ 14 kb.**

Principle

ReverTra Ace® is a high efficient M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase that has been genetically modified to remove RNase H activity and increase reaction efficiency. It is the preferred enzyme for applications requiring full-length cDNAs and high product yields from total RNA, mRNA, rRNA, etc.

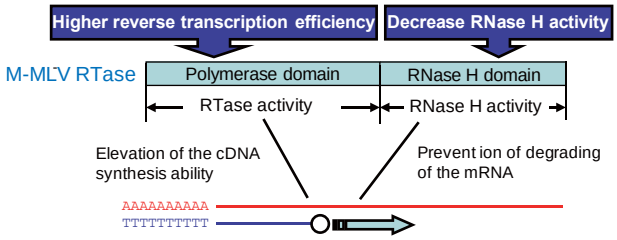


Fig. Genetically improved M-MLV Reverse transcriptase

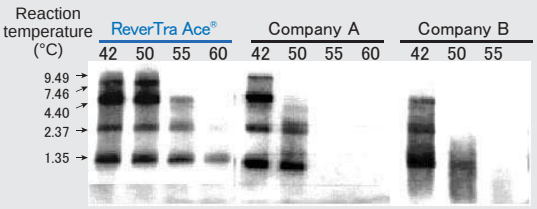


Fig. Comparison of elongation capability of RNase H minus RTases at various temperatures

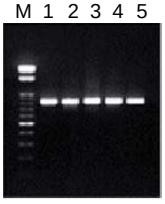
Applications

- cDNA synthesis
  (for RT-PCR , Library construction etc.)

Application data

1. Detection of human β-actin mRNA by RT-PCR utilizing ReverTra Ace -α-® and various PCR enzymes

cDNA was synthesized using 1 mg total RNA from the human cell line (HeLa cell) at 42°C for 20 minutes, followed by inactivation with ReverTra Ace -α-® in a volume of 20 μl at 99°C for 5 minutes. Subsequently, using the attained cDNA, the target gene (β-actin: 838 bp) was amplified with various PCR enzymes.The target genes were successfully amplified using the various PCR enzymes tested.



M: 100 bp Ladder Marker
1: rTth DNA polymerase
2: rTaq DNA polymerase
3: High efficient Taq DNA polymerase (Company A)
4: High efficient Taq DNA polymerase (Company B)
5: KOD Dash [Code No. LDP-101]

ReverTra Ace® qPCR RT Kit & Master Mix

ReverTra Ace® qPCR RT Kit (Code No. FSQ-101) is an efficient and convenient kit to synthesize high quality cDNAs for real-time PCR. This kit contains the highly efficient reverse transcriptase "ReverTra Ace® and a RT buffer optimized for the highly efficient synthesis of short-chain cDNAs suitable for real-time PCR. The protocol is simple, and the reaction can be completed in 15 min.

ReverTra Ace® qPCR RT Master Mix (Code No. FSQ-201) is an efficient and convenient reagent to synthesize high quality cDNAs for real-time PCR. The master mix reagent (5x) contains the highly efficient reverse transcriptase "ReverTra Ace®" , primers and buffer optimized for highly efficient synthesis of short-chain cDNAs suitable for real-time PCR. The protocol is simple, and the reaction can be completed in 15 min.

Code No. FSQ-101 200 reactions [10 µl per reaction]

Store at -20 °C

Components:

5x RT Buffer	400 µl
Enzyme Mix	100 µl
Primer Mix	100 µl
Nuclease-free water	2 x 1000 µl

Code No. FSQ-201 200 reactions [10 µl per reaction]

Store at -20 °C

Components:

5x RT Master Mix	400 µl
5x RT Maser Mix no RT-Control	40 µl
Nuclease-free water	2 x 1000 µl

Features

- **The optimized RT buffer and Primer Mix (oligo dT & random primers) enable highly efficient reverse transcription.**
- **The reaction can be completed in 15 min. The protocol does not contain an additional RNase H treatment step to remove residual RNA after reverse transcription (Patent Pending).**
- **Suitable for the detection of low abundance mRNAs. Since the RT buffer is optimized for real-time PCR, the addition of 20% (v/v) of the synthesized cDNA solution to the PCR solution does not inhibit the PCR reaction.**

Applications

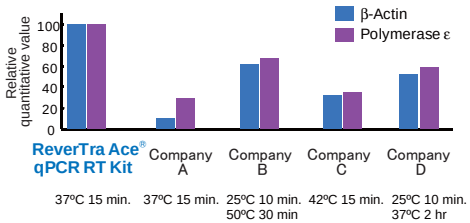
- cDNA sysnthesis for real-time PCR

Application data

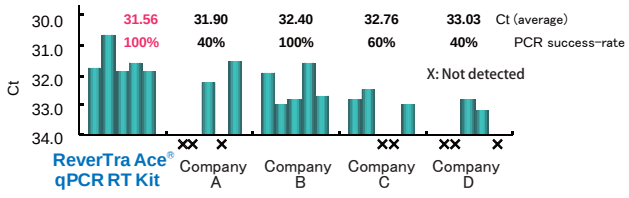
1. Comparison of cDNA yields using various reverse transcription kits

cDNA was synthesized from human cell line (HeLa cell) total RNA with the ReverTra Ace® qPCR RT Kit. Subsequently, quantification of β-actin and polymerase ε mRNA were performed by real-time PCR with 100 ng cDNA, in conjunction with the SYBR® Green I real-time PCR master mix.

As shown in the figure, the relative cDNA yields from the ReverTra Ace® qPCR RT Kit were greater than from the other commercial kits.



2. Detection of a rare expressing gene



Several 1-µl cDNA aliquots were prepared from 100 ng human cell line total RNA in a volume of 20 µl using various reverse transcription kits. Subsequently, a low expressing gene (TNF-α) was detected with the SYBR® Green I real-time PCR master mix.

The Ct values and success rates of the ReverTra Ace® qPCR RT Kit were greater than those from the other commercial kits.

ReverTra Ace® qPCR RT Master Mix with gDNA Remover

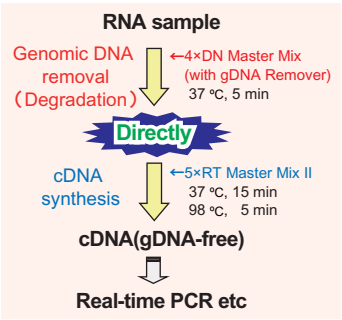
Code No. FSQ-301 200 reactions
[10 µl per reaction]

Store at -20 °C

Components:

gDNA Remover	10 µl
4x DN Master Mix	440 µl
5x RT Master Mix II	400 µl
5x RT Master Mix II no RT-Control	40 µl
Nuclease-free water	2 x 1000 µl

This kit includes master mix reagents for reverse transcription and for the removal of genomic DNA [DNase I treatment].
In many cases, total RNA prepared using spin-columns or acid guanidium-phenol- chloroform (AGPC) extraction methods contains small amount of genomic DNA. Any contaminating genomic DNA will be amplified along with cDNA, especially when primer pairs are designed within the same exon or from pseudogenes. Amplification from genomic DNA can result in qualitative and quantitative inaccuracies. The protocol consists of i) a genomic DNA degradation step using "gDNA remover" and ii) a reverse transcription step. The two steps can be achieved sequentially without purification or heat inactivation of DNase I.



Features

- **"Genomic DNA degradation step" and "cDNA synthesis step" can be achieved sequentially in approximately 30 min.**
- **The master mix reagent contains random and oligo dT primers optimized for efficient reverse transcription.**
- **Control, no reverse transcription experiments (no RT-Control) can be performed with 5x RT Master Mix II no-RT control.**

Applications

- cDNA sysnthesis for real-time PCR

Application data

1. Efficiency of genomic DNA removal

In Experiment 1, reverse transcription was performed accordint to the following condition and Table I. Then β-actin genes were detected by SYBR® Green I assay. No signal for the "C experiment" indicates that the contaminating genomic DNA in the RNA template was completely removed by "gDNA remover" (Fig. 1).
In Experiment 2, total RNA contain 100 ng genomic DNA were used as templates of reverse transcription uing various cDNA synthesis kits bearing the gDNA removal function and real-time PCR analysis was performed using the condition of Experiment 1. FSQ-301 showed the most sensitive and specific results (Fig. 2).

● cDNA synthesis
Template:
<Experiment 1> HeLa total RNA 0.5 µg /10 µl reaction
<Experiment 2> HeLa total RNA (0, 1pg, 10pg, 100pg, 1ng, 10ng,100ng, 1µg)
+human gDNA 100ng/20 µl reaction

● Real-time PCR
Reagent: THUNDERBIRD® SYBR® qPCR Mix (Code No.QPS-201)
Template: cDNA 2 µl/20 µl reaction (cDNA solution: 10%)
Target: β-actin (188 bp)
Real-time cycler: Applied Biosystems 7900HT

<<Experiment 1>>

Table I

	4×DN Master Mix	5×RT Master Mix
A	gDNA Remover (-)	RTase (-)
B	gDNA Remover (-)	RTase (+)
C	gDNA Remover (+)	RTase (-)
D	gDNA Remover (+)	RTase (+)

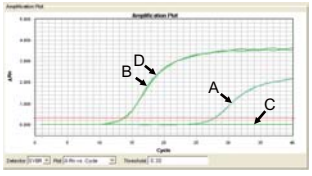


Fig. 1

<<Experiment 2>>

ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Code No. FSQ-301)

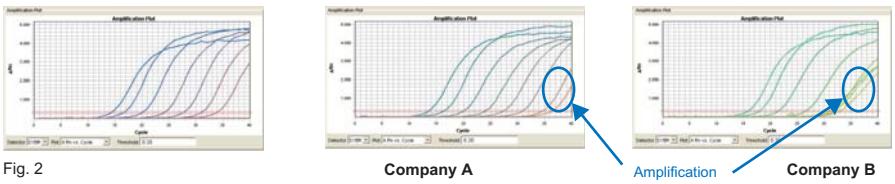


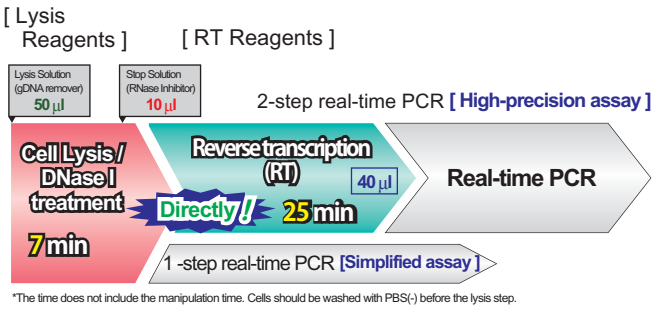
Fig. 2

Company A

Amplification from genomic DNA

Company B

SuperPrep® Cell Lysis & RT Kit for qPCR



*The time does not include the manipulation time. Cells should be washed with PBS(-) before the lysis step.

SuprePrep® Cell Lysis & RT Kit for qPCR (Code No. SCQ-101) consists of "Lysis Reagents" and "RT Reagents" for synthesis of cDNA templates for real-time PCR assays.
"Lysis Reagents" can be used to prepare cell lysates containing RNAs that can be used as templates for reverse transcription. "RT Reagents" can be used to carry out reverse transcription, and are optimized for efficient cDNA synthesis from crude lysates. The synthesized cDNA can be used as a template for two step real-time PCR. The cell lysate prepared by "Lysis Reagents" can be applied to one-step real-time PCR.

Code No. SCQ-101 100 reactions
Code No. SCQ-201 100 preparations

Store at -20 °C

Components:

SuperPrep® Cell Lysis & RT Kit for qPCR (Code No. SCQ-101) comprises the following reagents:

[Lysis Reagents]

− Lysis Solution	5.5 ml
− gDNA Remover	33 µl
− Stop Solution	1.1 ml
− RNase Inhibitor	55 µl

[RT Reagents]

− 5 x RT Master Mix	860 µl
− 5 x RT Master Mix no-RT control *	86 µl
− Nuclease-free Water	2 x 1.7 ml

*The master mix does not contain the reverse transcriptase and can be used in the control experiments.

SuperPrep® Cell Lysis Kit for qPCR (Code No. SCQ-201) is an option of "Lysis Reagents" . The cell lysate prepared by "Lysis Reagents" can be applied to one-step real-time PCR.

Features

RNA purification is not necessary.

Cell lysates can be prepared as effective templates for cDNA synthesis, reducing the total assay time.

High-quality cDNA can be obtained from cell lysates.

The optimized lysis solution efficiently inhibits RNA degradation during treatment. RNA in the lysate is stable on ice for at least 2 h. High-quality cDNA can be synthesized using highly efficient reverse transcriptase "ReverTra Ace®" with low contamination of genomic DNA because of preceding DNase I treatment. The reverse transcriptase is supplied as a master mix reagent containing optimally mixed primers (random and oligo dT) to achieve effective cDNA synthesis.

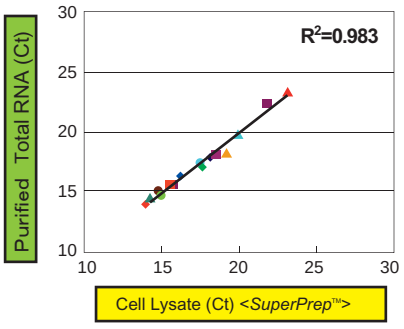


Fig. Comparison of the efficiency with purified RNA

SuperPrep® Cell Lysis & RT Kit for qPCR (Code No SCQ-101) synthesized cDNA using the lysate from 2.5 x 10^4 HEK293 cells in a 40 µl reaction. cDNA was synthesized using 66.6 ng of total RNA, corresponding to 2.5 x 10^4 HEK293 cells, using a cDNA synthesis kit (ReverTra Ace® qPCR RT Master Mix [Code No. FSQ-201]) in a 40 µl reaction. Fifteen housekeeping genes were then analyzed by SYBR® Green real-time PCR using the synthesized cDNA. High correlation was observed between the two methods.

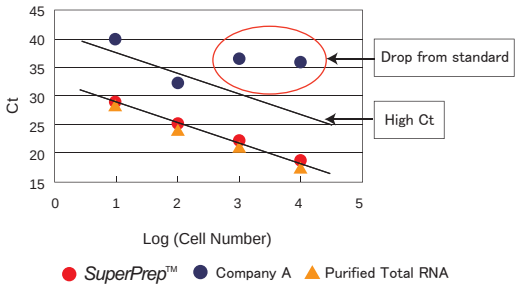


Fig. Evaluation of efficiency for expression analysis of cells with high RNase activity

Expression analysis using a lysate from U937 cells is difficult because of high RNase activity. In this experiment, the lysates (8 µl) from 1x10^4, 1x10^3, 1x10^2 and 1x10^1 cells were used. β-actin genes were analyzed by SYBR® Green real-time PCR assay using cDNA synthesized from the lysates in 40 µl reaction. The same experiments were performed using two similar systems (*SuperPrep*® and another company' s product [Company A]) and conventional method using corresponding amounts of RNAs. *SuperPrep*® showed successful amplification, comparable to the conventional method.

Reduction of dispersion on high-throughput assay.

Decreasing the small-volume dispensing and dilution steps from the protocol reduced the dispersion of data in a high-throughput assay. Integrating or omitting some steps improved the usability. Cells can be lysed on the culture plate without pipetting, and DNase I treatment can be performed at the same time as the lysis step. The DNase I reaction can be stopped by adding "Stop Solution" . Additional purification or heating steps are not necessary.

Table Cells tested by this system

	Cell Name	Adherent/Non-adherent	Species	Remarks
1	A431	Adherent	*H.sapiens*	epidermoid carcinoma cell line
2	C_2C_{12}	Adherent	*M.musculus*	myoblast cell line
3	Caco-2	Adherent	*H.sapiens*	colon adenocarcinoma cell line
4	CHO-K1	Adherent	*C.griseus*	ovary cell line
5	COLO205	Non-adherent	*H.sapiens*	colon adenocarcinoma cell line
6	DLD-1	Adherent	*H.sapiens*	colon adenocarcinoma cell line
7	HCT-15	Adherent	*H.sapiens*	colon adenocarcinoma cell line
8	HDF	Adherent	*H.sapiens*	primary foreskin fibroblasts (primary cell)
9	HEK293	Adherent	*H.sapiens*	embryonic kidney cell line
10	HeLa S3	Adherent	*H.sapiens*	cervix carcinoma cell line
11	HepG2	Adherent	*H.sapiens*	hepatocellular carcinoma cell line
12	HUVEC	Adherent	*H.sapiens*	umbilical vein endothelial cells (primary cell)
13	Jurkat	Non-adherent	*H.sapiens*	T lymphocyte cell line
14	K562	Non-adherent	*H.sapiens*	myelogenous leukemia cell line
15	KUSA-A1	Adherent	*M.musculus*	bone marrow stromal stem cell line
16	L929	Adherent	*M.musculus*	aneuploid fibrosarcoma cell line
17	MCF7	Adherent	*H.sapiens*	breast adenocarcinoma cell line
18	Neuro2a	Adherent	*M.musculus*	neuroblastoma cell line
19	NIH-3T3	Adherent	*M.musculus*	embryo fibroblast cell line
20	PC12	Adherent	*R. norvegicus*	adrenal pheochromocytoma cell line
21	rMSC	Adherent	*R. norvegicus*	bone marrow stromal stem cells (primary cell)
22	THP-1	Non-adherent	*H.sapiens*	acute monocytic leukemia cell line
23	U937	Non-adherent	*H.sapiens*	leukemic monocyte lymphoma cell line

- **Various real-time PCR reagents can be applied.**

The synthesized cDNA can be used in various real-time PCR assay (TaqMan® probe, SYBR® Green etc).
In addition, the cell lysate can be applied to one-step real-time PCR reagents.

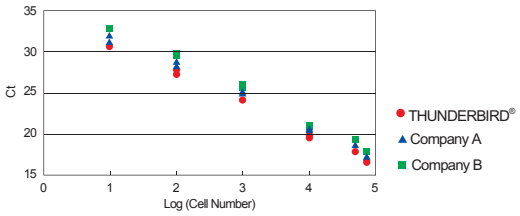


Fig. TaqMan® Probe assay using cDNA synthesized from cell lysates prepared by *SuperPrep*®.

cDNAs were synthesized using the cell lysates (8 μl) prepared from $7.5x10^4$, $5x10^4$, $1x10^4$, $1x10^3$, $1x10^2$ and $1x10^1$ HeLa S3 cells by *SuperPrep*® in 40 μl reaction. β-actin genes were detected by various real-time PCR reagents with TaqMan® real-time PCR assay. Successful amplifications were obtained from all reagents tested and the THUNDERBIRD® Probe qPCR Mix tended to show a better Ct than the other tested methods.

Applications

- cDNA sysnthesis for real-time PCR from mammalian cultured cells

Application data

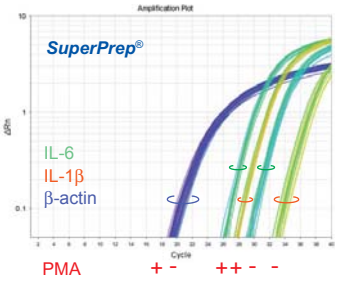
1. Evaluation of the assay variation

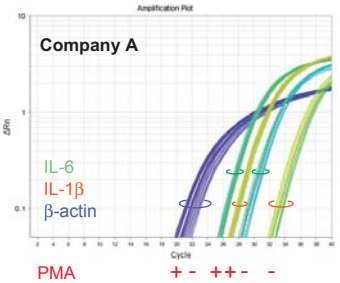
HeLa S3 cells were incubated with or without 100 nM phorbol 12-myristate 13-acetate (PMA) for 24 h after seeding at 2 $x10^4$ cells/well in a 96-well culture plate. cDNA were synthesized from the lysates prepared from the cells washed with PBS(-).IL-6, IL-1β and β-actin genes were detected by TaqMan® real-time PCR assay with THUNDERBIRD® Probe qPCR Mix (Code No. QPS-101). After compensation of the Cts of IL-6 and IL-1β by that of β-actin, the ΔΔCt between with or without PMA and Z' factors* were calculated.
Z' factors from *SuperPrep*® were superior to that from the other system (Company A).

∗Z'-factor : The Z' factor is a simple statistical parameter that is used to assess the quality of high-throughput screening (HTS) assays. A Z' score of ≥0.5 is generally considered to indicate good quality Z' can be calculated by the following formula.

$$Z' = 1-3 \times [\Delta Ct(+) \text{ standard deviation} + \Delta Ct(-) \text{ standard deviation}]/|\Delta\Delta Ct|$$

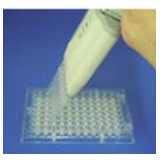
<Amplificsation curve>





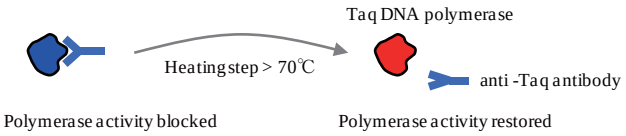
<IL-6>	PMA	Ct (IL-6) Mean	Δ Ct (IL-6 - β-actin) Mean	S.D.	Δ Δ Ct	Z'
SuperPrep™	(+)	26.96	6.27	0.14	-3.85	0.62
	(-)	30.24	10.12	0.35		
Company A	(+)	26.50	4.30	0.34	-3.88	0.59
	(-)	29.44	8.18	0.19		

<IL-1β >	PMA	Ct (IL-1β) Mean	Δ Ct (IL-1β - β-actin) Mean	S.D.	Δ Δ Ct	Z'
SuperPrep™	(+)	28.62	7.93	0.15	-5.94	0.74
	(-)	33.99	13.87	0.38		
Company A	(+)	28.00	5.80	0.47	-6.19	0.61
	(-)	33.26	11.99	0.34		



anti-Taq DNA polymerase antibody

anti-Taq high



anti-Taq high is a highly purified neutralization monoclonal antibody to Taq DNA polymerase. This product provides an antibody-mediated hot start PCR to enhance the specificity and sensitivity of PCR. This antibody inhibits polymerase activity before the onset of thermal cycling, preventing primer dimer formation and non-specific amplification. At the first denaturation step of the thermal cycling, the antibody is quickly inactivated and PCR proceeds. The antibody-mediated hot start method is significantly more convenient to use than other hot start methods.

Code No. TCP-101 100 µl

<100 µl of anti-Taq high corresponds to 500 U Taq DNA polymerase>

Store at -20 °C

Components:

anti-Taq high (1 µg/µl)*	100 µl
10 x PCR buffer	1 ml

* Storage buffer: 10 mM Tris-HCl (pH 7.0), 50 mM KCl, 50% Glycerol

Taq DNA polymerase* 1 U
anti-Taq high 0.2 µl

or → Room temperature, 5min**

Taq DNA polymerase* (2.5 U/µl)
Equal volume of anti-Taq high

* Taq-based high efficient PCR enzymes can be used.
**The polymerase-antibody mixture can be stored for long term at -20℃.

Features

- **Enhances the specificity and sensitivity of PCR.**
- **Inhibits ≥95% of polymerase activity at 45ºC.**
- **No contamination of mouse genomic DNA, as determined PCR.**
- **The polymerase can be reactivated quicker than with methods utilizing a chemically modified polymerase.**

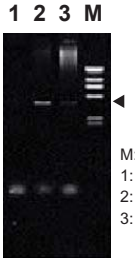
Applications

- Antibody-mediated hot start PCR

Application data

1. Application of the hot start PCR using a Taq based high efficient PCR enzyme

The efficiency of anti-Taq antibodies were evaluated by the amplification of the human β-globin gene (3.6 kb). The result indicates that anti-Taq high increases the specificity and sensitivity of the PCR compared with the control reaction and PCR mediated hot start using company A's anti-Taq antibody.



M: λ/*Hind* III Marker
1: Taq-based high efficient PCR enzyme
2: Taq-based high efficient PCR enzyme + anti-Taq high
3: Taq-based high efficient PCR enzyme + anti-Taq antibody (company A)

Recombinant type RNase inhibitor

RNase inhibitor

This reagent is a recombinant type human ribonuclease inhibitor purified using a combination of ion exchange and affinity chromatography.
This inhibitor exhibits broad-spectrum RNase inhibitory properties, including RNase A, RNase B, RNase C and human placental RNase. Does not inhibit RNase T1, S1 nuclease, RNase from *Aspergillus*, and RNase H. The 50kDa protein exerts its inhibitory effect by noncovalently binding to RNases in a 1:1 ratio. This inhibitor can be applied to a reverse transcriptase reaction.

Code No.SIN-201 2,500U

Store at -20 °C

Components:

RNase inhibitor (20-40 U/µl)*
* Specific activity : approximately 100 U/µg protein

Unit definition

One unit is defined as the amount of RNase inhibitor required to inhibit the activity of 5 ng of ribonuclease A by 50%.

dNTPs Mixture (2 mM)
dNTPs Mixture (10 mM)
dNTPs Set

dNTPs Mixture is an equal moler solutoin mixture of ultrapure dATP, dCTP, dGTP and dTTP. dNTPs Set contains each dATP, dCTP, dGTP and dTTP solution.

Applications

- PCR
- Reverse transcription

Code No.
dNTPs Mixture (2mM) : NTP-201
dNTPs Mixture (10mM) : NTP-301
dNTPs Set : NTP-101

Store at -20 °C

Components:

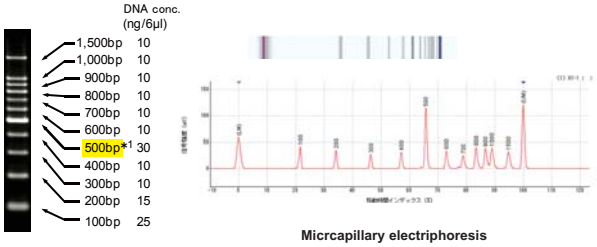
[NTP-201]
dNTPs Mixture (2 mM), 1 ml <2 µmoles/1ml>

[NTP-301]
dNTPs Mixture (10 mM), 0.2 ml <2 µmoles/0.2 ml>

[NTP-101]
dATP, dGTP, dCTP, dTTP (100 mM), 0.5 ml each
<4 x 50 µmoles/0.5 ml>

100 bp DNA Ladder

A 100 bp DNA Ladder is suitable for use as a molecular weight standard (100 bp~1,500 bp) for agarose gel electrophoresis. A 500 bp fragment* has increased intensity as a reference band. Dye-attached and dye-mixed versions (Loading Quick®) are available.



Micrcapillary electriphoresis

Code No.
DNA-035 15 µg(100 gel lanes)
DNA-135 15 µg(100 gel lanes) Loading Quick®

Store at -20 °C

Components:

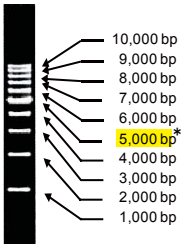
[DNA-035] <0.03 µg/µl>
DNA ladder marker solution 500 µl
6 x Loading Dye 1 ml

[DNA-135] <0.025 µg/µl> [dye-attached version]
DNA ladder marker solution 600 µl

Features

- **Dye-attached and dye-mixed versions (Loading Quick®) are available**
- **The 500 bp fragment is enhanced**
- **The DNA mass in each band is determined. The approximate DNA mass in each bands in the 100 bp Ladder is as above (assuming a 0.15 µg loading):**

1 Kb DNA Ladder



A 1kb DNA Ladder is suitable for use as a molecular weight standard (1,000 bp~10,000 bp) for agarose gel electrophoresis. A 5,000 bp* fragment has increased intensity as a reference band.

Code No. DNA-032 90 µg (300 gel lanes)

Store at -20 °C

Components: <0.3 µg/µl>

DNA ladder marker solution 300 µl
6 x Loading Dye 1 ml

Features

- **Dye for electrophoresis is attached to the product.**
- **The 5 kb fragment is enhanced.**

TArget Clone™/ TArget Clone™ -Plus-

TArget Clone™ and TArget Clone™ -Plus- are high efficient TA cloning kits. TArget Clone™ [Code No. TAK-101] can be applied to the TA cloning of PCR products amplified using Taq DNA polymerase, Blend Taq® [Code No. BTQ-101], Blend Taq® -Plus- [Code No. BTQ-201] or KOD Dash [Code No. LDP-101]. Almost all PCR products having dA overhanging at the 3' end are available. TArget Clone™ -Plus- [Code No. TAK-201] can be applied to the TA cloning of blunt-end PCR products amplified using KOD -Plus- series or KOD FX series. TArget Clone™ -Plus- contains 10xA-attachment mix. This reagent is a mixture of anti-KOD DNA polymerase antibody specific to KOD 3'→5' exonuclease activity (proof-reading activity) and Taq DNA polymerase, which exhibits terminal transferase activity. The 10 x A-attachment mix allows for blunt end PCR products to acquire overhanging dA at the 3' -ends.

Code No. TAK-101 (TArget Clone™) **10 reactions**
TAK-201 (TArget Clone™ -Plus-) **10 reactions**

Store at -20 °C

Components:

<TAK-101>	
pTA2 Vector (50 ng/μl)	10 μl
2x Ligation Buffer	50 μl
T4 DNA Ligase	10 μl
<TAK-201>	
pTA2 Vector (50 ng/μl)	10 μl
2x Ligation Buffer	50 μl
T4 DNA Ligase	10 μl
10x A-attachment Mix	10 μl
<TAK-301>	
10x A-attachment Mix	25 μl

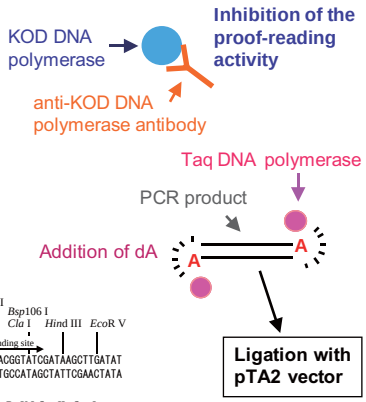
Features

- **PCR products can be used without purification.**
- **Ligation step can be completed in 5 min.**
- **Addition of dA to the 3'-ends of PCR products can be competed in 10 min at 60ºC [10XA-attachment Mix].**
- **The vector generates blue *E. coli* colonies on X-gal / (IPTG) plates in the absence of insert.**

Applications

- TA Cloning

Product Name	Applicable for PCR products amplified with
TArget Clone™	Taq DNA polymerase, Blend Taq®, Blend Taq® -Plus-, KOD Dash <PCR products having overhanging dA at the 3' -ends>
TArget Clone™ -Plus-	KOD -Plus- series, KOD FX series



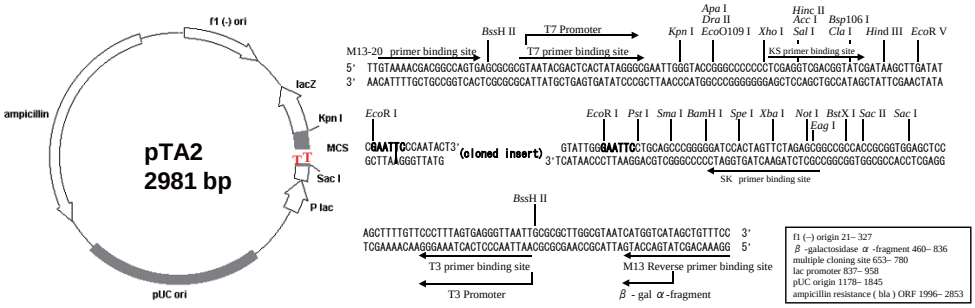
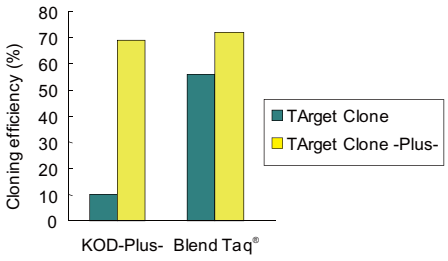


Fig. Map of pTA2 vector and multiple cloning site (MCS)

Application data

1. TA cloning of a 500 bp-PCR product amplified with KOD -Plus- and Blend Taq®

Human β-globin gene (0.5 kb) was amplified using KOD -Plus- [Code No. KOD-201] or Blend Taq® [BTQ-101]. Then, the unpurified PCR products were cloned into the pTA2 vectors using TArget Clone™ or TArget Clone™ -Plus- according to each instruction manual. Subsequently, DH5α competent cells were transformed using the ligation products and cultured on the LB/Amp/X-gal plate overnight at 37℃.
PCR products from Blend Taq® were successfully cloned into the pTA2 Vector using both kits. And, PCR products from KOD -Plus- was cloned efficiently into pTA2 Vector using TArget Clone™ -Plus-.



Site-directed mutagenesis kit

KOD -Plus- Mutagenesis Kit

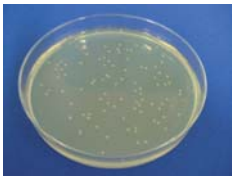
Code No. SMK-101　20 reactions

Store at -20 °C

Components:

KOD -Plus-（1 U/μl）	25 μl
10x Buffer for iPCR	125 μl
2 mM dNTPs	125 μl
Dpn I (10 U/μl)	50 μl
T4 Polynucleotide Kinase (5 U/μl)	50 μl
Ligation high (T4 Ligase + Buffer Mixture)	250 μl
Control Plasmid pAK119M (50 ng/μl)	10 μl
Control Primer #1 (10 pmol/μl)	10 μl
Control Primer #2 (10 pmol/μl)	10 μl

This kit is an inverse PCR (iPCR)-based site-directed mutagenesis kit using KOD DNA polymerase (1)(2) as a high-fidelity PCR enzyme. This reagent was developed based on a high efficient and efficient PCR reagent, "KOD -Plus- (Code No. KOD-201)", which consists of KOD DNA polymerase and anti-KOD DNA polymerase antibodies(3) for Hot Start PCR.
This kit enables not only the introduction of point mutations, but also the introduction of large insertions and deletions. The PCR fidelity of KOD -Plus- is greater than Taq DNA polymerase (ca. 80-fold); therefore, unexpected, 2nd-site mutations can be reduced. PCR reactions can be performed using standard PCR primers and do not require phosphorylated primers, because this protocol contains a 'Phosphorylation Step' of PCR products.



Features

- **Applicable for various mutations, such as substitutions, insertions, and deletions.**
- **High efficiency (95% maximum) can be obtained.**
- **Simple protocol facilitates speedy experiments. Phosphorylated primers are not required.**

Applications

- Site-directed mutagenesis
  - Substitution (*e.g.* Point mutation, Point mutation library)
  - Deletion (*e.g.* 1 bp – several kbp deletion)
  - Insertion (*e.g.* Introduction of His-tag sequence)

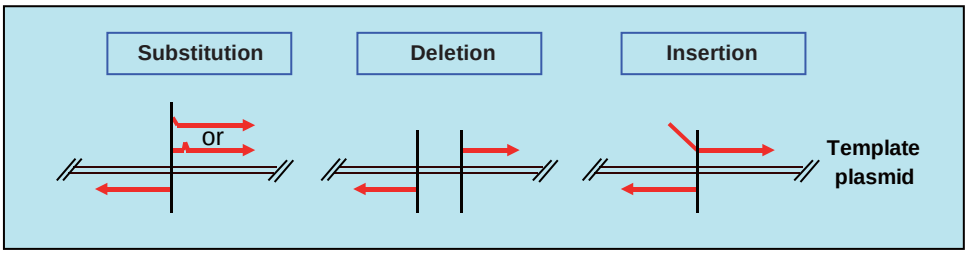
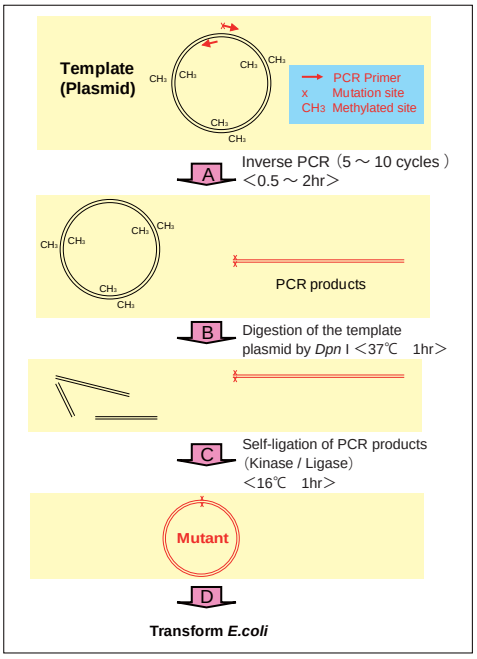


Fig. 1 Typical site-directed mutagenesis

References

1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.*, 63: 4504-10 (1997)
2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.*, 306: 469-77 (2001)
3) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem.*, 126: 762-8 (1999)

Principle



A) Inverse PCR of plasmid DNA, using a mutation primer.

B) Plasmid DNA is digested by *Dpn* I.

Note: *Dpn* I digests methylated DNA, such as plasmid DNA from typical *E. coli* cell lines (e.g. JM109 and DH5α).

C) Self-ligation of PCR products is performed by a reaction with T4 polynucleotide kinase and ligase.

D) Transformation of *E. coli* cell lines using self-ligated PCR products.

Fig.2 Flow chart of KOD -Plus- Mutagenesis Kit

Application data

1. Comparison of the efficiency on deletion and insertion experiments

A 90 bp DNA sequence was deleted from a plasmid carrying FLJ32066 gene (7.3 kb) using KOD -Plus- Mutagenesis Kit [Code No. SMK-101] and compared with a kit from an alternative company (company A). In parallel, 18 bp-DNA cording a 6×His-tag sequence was inserted into the plasmid. As shown in Tables 1 and 2, the expected mutants were successfully obtained using the KOD -Plus- Mutagenesis Kit.

Table 1. Results of the mutation experiments for deletion of a 90 bp sequence

	Number of clones tested	Number of clones with mutations	Expected percentage of clones with the mutation
KOD -Plus- Mutagenesis Kit	16	14	88%
Company A (non-PCR method)	16	7	44%

Table 2. Results of the mutation experiments for insertion of an 18 bp sequence (6xHis-tag sequence)

	Number of clones tested	Number of clones with mutations	Expected percentage of clones with the mutation
KOD -Plus- Mutagenesis Kit	16	15	94%
Company A (non-PCR method)	16	0	0%

2. Frequency of the unexpected mutation (2nd mutation)

A 2,000 bp region in the plasmids from mutated clones was obtained from a control reaction (8 cycles) using Control plasmid (4.3 kb), Control primer #1, and Control primer #2 attached with KOD -Plus- Mutagenesis Kit [Code No. SMK-101] were sequenced. As shown in Table 1, the number of unexpected mutations was found to be approximately 1 base per 48,000 bases.

Table 1. Frequency of unexpected mutations (2nd mutation)

	Expected mutation rate (%)	Number of clones tested	Total number of bases sequenced	Number of unexpected mutations (2nd mutation)	Rate of unexpected mutation (2nd mutation) [$X10^{-3}$](%)
KOD -Plus Mutagenesis Kit	>80%	24	48,000	1	2.08
Company A (non-PCR method)	>80%	24	48,000	2	4.16

Efficient master mix for ligation

Ligation high

Ligation high is a highly efficient premixed T4 Ligase reagent. The reagent enables repid and effective ligation.

Code No. LGK-101 2 x 375 µl

Store at -20 °C

Components:

Ligation high	2 x 375 µl

Features

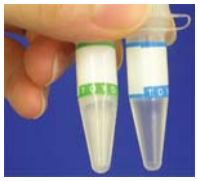
- **Effective ligation of cohesive, blunt and A overhang DNA fragments can be achieved.**
- **Simply mix Ligation high with an equal volume or with double the volume of the solution containing DNA fragments.**

Applications

- Ligation of DNA frangments

Efficient master mix for ligation

Ligation high Ver.2



State of the reagents at -20 °C
Left Ligation high
Right: Ligation high Ver.2

Ligation high Ver.2 is an improved efficient premixed T4 Ligase reagent. The reagent enables repid and effective ligation. The reagent will not freeze at -20 °C.

Code No. LGK-201 750 µl

Store at -20 °C

Components:

Ligation high Ver.2	750 µl

Vector DNA＋Insert DNA	7.5 µl
Ligation high Ver.2	3.75~7.5 µl
16°C, 30 min.	

Features

- **The ligation effieciency for A overhang DNA fragments is improved.**
- **Will not freeze at -20ºC. No need to thaw.**
- **Simply mix Ligation high Ver.2 with an equal volume or with double the volume of the solution containing DNA fragments.**

Highly efficient blunting reagent

Blunting high

Blunting high is a kit that produces a blunt end at the DNA terminus, and allows for its use in a subsequent ligation step using KOD DNA polymerase and DNA ligase reagents, respectively.

Code No. BLK-101 20 reactions

Store at -20 °C

Components:

KOD DNA Polymerase (2.5 U/µl)	20 µl
10 x Blunting Buffer	100 µl
Ligation high	375 µl
Control DNA (10 ng/µl)	50 µl

Features

- **The blunting step makes use of KOD DNA polymerase, which exhibits polymerase and 3'→5' exonuclease activities.**
- **The blunting step is completed within 2 min.**
- **A highly efficient premixed ligation reagent, "Ligation high" is included in this kit.**

Applications

- Blunting and ligation

Thermo T7 RNA Polymerase

Thermo T7 RNA polymerase is a genetically modified T7 RNA polymerase exhibiting increased thermal stability. The optimum reaction temperature of this enzyme is around 50 ºC. The half-life of the enzyme at 50 ºC is approximately 85 min.

Code No. TRL-201 7,500 U

Store at -20 °C

Components:

Thermo T7 RNA polymerase (50 U/µl)	150 µl
10 x Buffer	2 x 1ml

* The following reagents are not supplied;
NTP and RNase inhibitor.

Features

- **Exhibits greater specific activity than wild type enzyme at 37-50ºC.**

Applications

- RNA probe preparation
- RNA synthesis for *in vitro* translation
- RNA synthesis for RNA splicing studies
- Capped mRNA synthesis using a cap analogue

Unit definition

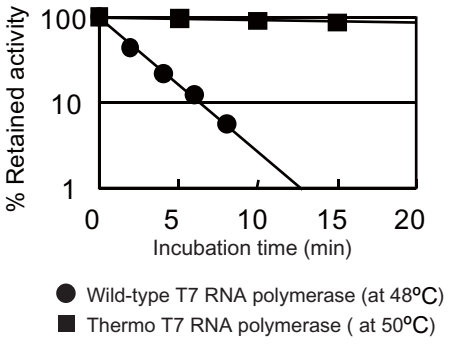
One unit is defined as the amount of enzyme that will incorporate 1 nmole of [^{3}H] rNTP into an acid insoluble material using T7 DNA as template in 60 min at 37ºC.

Application data

1. Comparison of heat stability

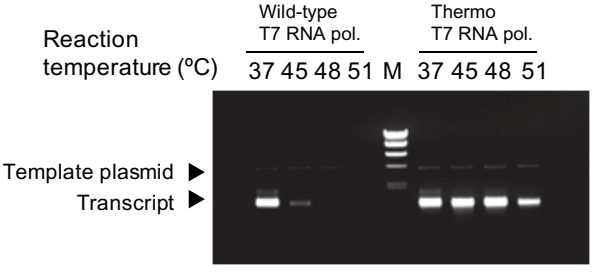
The residual activities of the wild-type T7 RNA polymerase and Thermo T7 RNA polymerase were measured after incubation for various periods at 48ºC and 50ºC, respectively. As a result, Thermo T7 RNA polymerase retained its activity after incubation for 15 min whereas the activity of wild-type enzyme decreased to 1/10 after incubation for 5 min.

The half-lives of the enzymes have been estimated to be:
Wild-type T7 RNA polymerase: < 1.9 min.
Thermo T7 RNA polymerase: 84.5 min.



2. *In vitro* transcription at high temperature

In vitro transcription activities were compared between wild-type and Thermo T7 RNA polymerases. Distinct transcripts were detected on an agarose gel from 37 to 51ºC with Thermo T7 RNA polymerase.

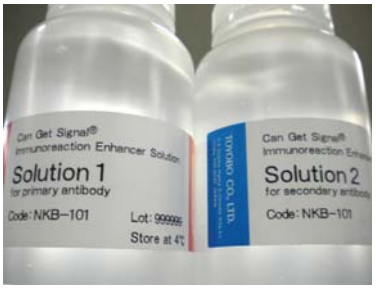


Template:
Linearlized plasmid DNA carrying T7 promoter, 0.5 µg

Reaction time: 60 min.

Immunoreaction accelerator for western blot & ELISA

Can Get Signal® Immunoreaction Enhancer Solution



Can Get Signal® is a solution containing an accelerator for antigen-antibody reactions. This reagent improves sensitivity, specificity, and signal-to-noise ratio (S/N) for western blot, dot blot, enzyme-linked immunosorbent assay (ELISA), etc. Solutions 1 and 2 refer to the reactions of the primary and secondary antibodies, respectively.

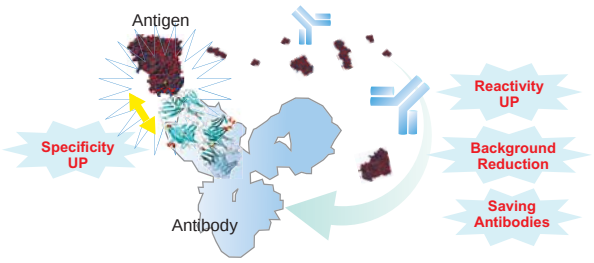
Code No.

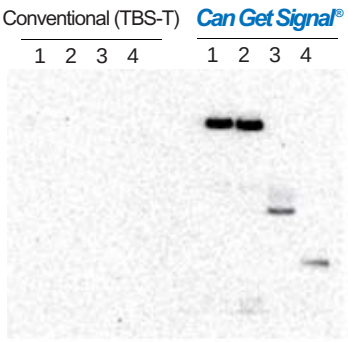
NKB-101	250 ml each
NKB-101T	50 ml each
NKB-201	250 ml
NKB-301	250 ml

Store at 4 °C

Components:

Reagent Name	Code No.			
	NKB-101T	NKB-101	NKB-201	NKB-301
Solution 1 for primary antibody	50 ml	250 ml	250 ml	-
Solution 2 for secondary antibody	50 ml	250 ml	-	250 ml





His-tagged recombinant proteins were detected with *Can Get Signal*® and a conventional method (TBS-T). *Can Get Signal*® showed excellent greater sensitivity than the conventional method.

1: 6xHis-Catalase fusion protein
2: 6xHis-Catalase fusion protein
3: 6xHis-c-fos
4: 6xHis-c-jun

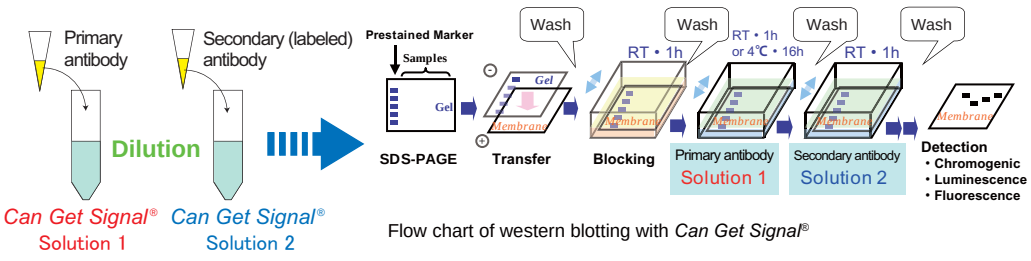
Fig. Detection of His-tagged proteins by Western blot.

Features

- **Enhances immunoassay signals up to several dozen times by maintaining low background signals.**
- **Can be used in combination with secondary antibodies labeled with peroxidase or alkaline phosphatase.**
- **Can be used directly without dilution (ready-to-use).**

Applications

- Western blot
- Dot blot
- ELISA

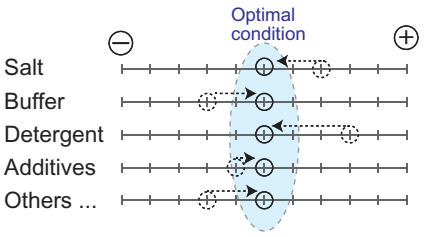


Flow chart of western blotting with *Can Get Signal*®

Principle

Our "immunoreaction enhancing technology (IE technology)" is based on acceleration of antigen-antibody reaction whilst keeping high specificity and low background by optimization of the antibody diluents. This technology is applied to the *Can Get Signal*® (Code No. NKB-101) and *Can Get Signal*® immunostain (Code No. NKB-401) series.

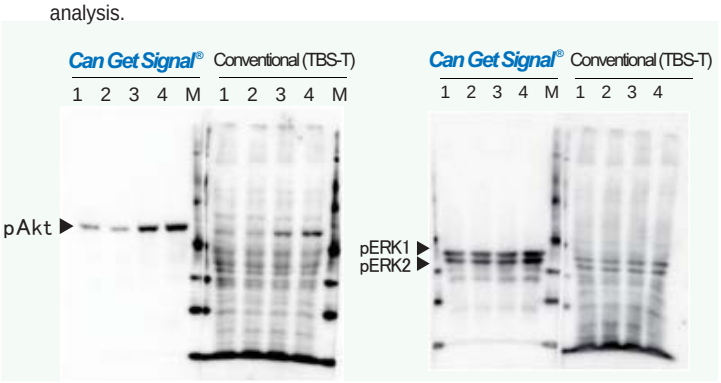
Can Get Signal® (Code No. NKB-101) improves sensitivity, specificity, and signal-to-noise ratio (S/N) for western blotting, dot blotting, and enzyme-linked immunosorbent assay (ELISA), etc.



Application data

1. Detection of phosphorylated proteins by western blotting

Phosphorylated Akt and ERK were detected by western blotting analysis using *Can Get Signal*® and a conventional method (TBS-T). As a result, the signal intensities of the target bands obtained with *Can Get Signal*® were greater than those of the conventional method. The background level of the experiment with *Can Get Signal*® was also significantly lower than that of the conventional method. The results suggest that *Can Get Signal*® improves the sensitivity and specificity of Western blotting analysis.



Sample：Cultured bovine adrenal medulla cells

1. Control (H_2O)
2. Insulin (1 nM, stimulated for 5 min)
3. Insulin (10 nM, stimulated for 5 min)
4. Insulin (100 nM, stimulated for 5 min)

Antibodies:

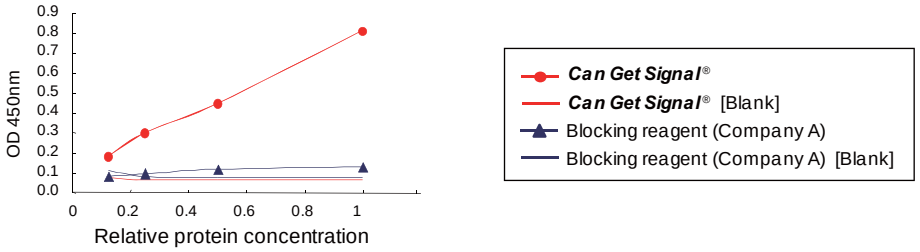
<p-Akt>
Primary antibody： Anti Phospho-Akt rabbit polyclonal antibody (1:2,000 dilutioin)
Secondary antibody：Anti rabbit-HRP antibody (1:20,000 dilution)

<p-ERK>
Primary antibody： Anti Phospho-ERK monoclonal antibody (1:2,000 dilutioin)
Secondary antibody：Anti mouse-HRP antibody (1:20,000 dilution)

Fig Detection of phosphorylated protein kinases (p-Akt, p-ERK1 and p-ERK2) by Western blotting with *Can Get Signal*® and a conventional method
* The data was kindly provided by Dr. Yanagita from the Department of Pharmacology, Faculty of Medicine, University of Miyazaki.

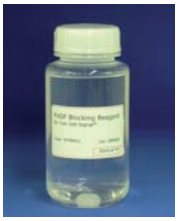
2. Detection of His-tagged proteins by ELISA

Sandwich ELISA (solid phase antibody: anti-ERK2 monoclonal antibody, primary antibody: anti-His tag polyclonal antibody, secondary antibody: anti-rabbit IgG-HRP antibody) was performed to detect his-tagged human MAP kinase (His-ERK2) synthesized by a cell-free protein synthesis system. *Can Get Signal*® showed an excellent quantitative curve as a function of antigen concentration whereas the conventional method with TBS-T resulted in low signals.



Blocking reagent for western blot analysis

PVDF Blocking Reagent for *Can Get Signal*®



PVDF Blocking Reagent for *Can Get Signal*® is a high performance blocking reagent optimized for western blot analysis. The reagent consists of a synthesized polymer, with no protein components. The reagent can be used efficiently with *Can Get Signal*®

*This reagent is manufactured by NOF CORPORATION.

Code No. NYPBR01 500 ml

Store at 4 °C

Applications

- Western blot
- Dot blot

Features

- **Optimized for use together with *Can Get Signal*® Immunoreaction Enhancer Solution (Code No. NKB-101) for western blot analysis.**
- **Suitable for detection of phosphorylated proteins because it does not contain any protein components.**
- **Unlike conventional blocking reagents (*e.g.*, non-fat milk and gelatin), minimize the masking effects of low signal intensities.**

Immunoreaction accelerator for IHC

Can Get Signal® immunostain

Immunoreaction Enhancer Solution

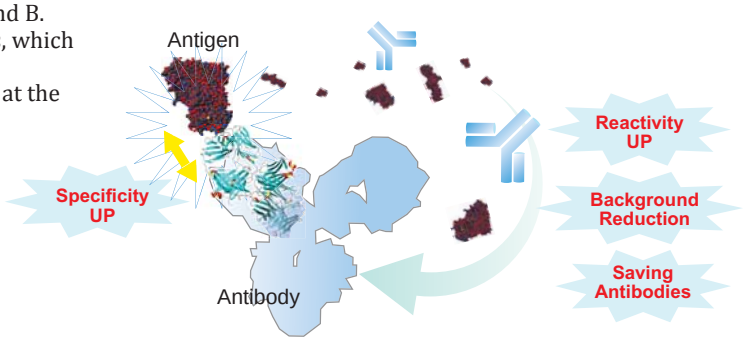
Can Get Signal® immunostain is a reaction solution that contains an accelerator for antigen-antibody reactions, which improves sensitivit y, specificity, and signal-to-noise ratio (S/N) of immunohistochemistry (IHC) and immunocytochemistry.
Can Get Signal® immunostain contains Solution A and B. These solutions exhibit various acceleration effects, which are antigen/antibody-dependent, and can be used independently. Both solutions should be examined at the begining.

Code No. **NKB-401** **5 ml each**
NKB-501 **20 ml**
NKB-601 **20 ml**

Store at 4 °C

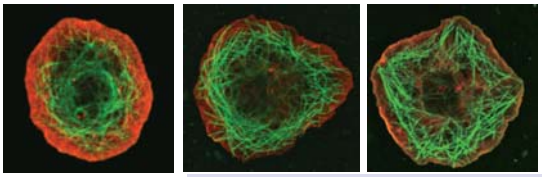
Components:

Reagent Name	Code No.		
	NKB-401	NKB-501	NKB-601
Solution A	5 ml	20 ml	-
Solution B	5 ml	-	20 ml



Features

- ***Can Get Signal***® **immunostain improves sensitivity, specificity, and signal to noise ratio of immunohistochemistry.**
- **This system can be applied to various detection systems (e.g., chromogenic, chemiluminescence, or fluorescence).**
- **The reagent can be used with ABC or polymer complex methods.**
- ***Can Get Signal***® **immunostain consists of Solution A and B, which exhibit various properties for improving results. These reagents can be used independently.**
- **The reagents can be used directly without dilution. <Ready-to-use type>**

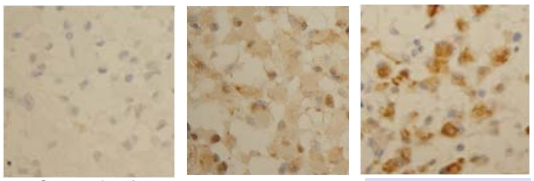


(1) Conventional (1% BSA, 0.02% TX-100 /PBS) | (2) *Can Get Signal*® immunostain (Solution A) | (3) *Can Get Signal*® immunostain (Solution B)

Fig. Detection of alpha-tubulin in Schneider 2 (S2) cells
Better results were obtained from solution A and B.

<Specimen> Drosophila culture cell line (Schneider 2 (S2) cells)
<Blocking> 1% BSA, 0.02% TX-100/PBS
<Primary antibody>
monoclonal anti-alpha-tubulin, antibody, mouse <Sigma>(1:100 dilutioin)

<Secondary antibody>
Alexa Fluor 488 goat anti-mouse (H+L) highly cross adsorbed
<Life Technologies> (1:600 dilution)
<Diluent>
(1) 1%BSA, 0.02% TX-100/PBS (Conventional)
(2) *Can Get Signal*® immunostain Solution A
(3) *Can Get Signal*® immunostain Solution B



Conventional (1% BSA/PBS) | *Can Get Signal*® immunostain (Solution A) | *Can Get Signal*® immunostain (Solution B)

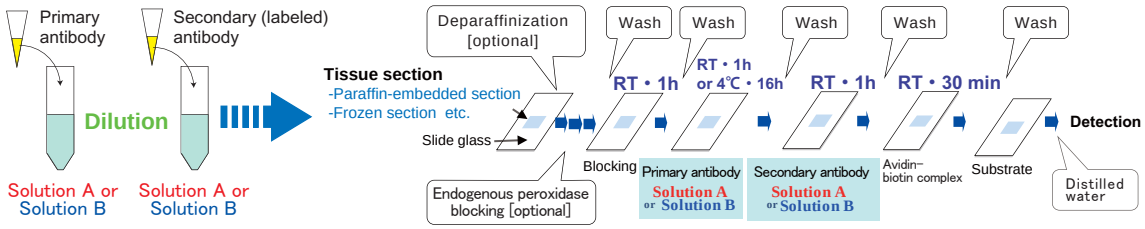
Fig. Detection of CD133 using paraffin embedded section
Better result was obtained from Soluton B.

<Specimen> Human brain cancer (glioblastoma)
<Sample preparation> Paraffin Embedded Section
<Blocking>
Incubated with 1%H_2O_2/methanol for 10 min and
Protein Block, Serum-free for 10 min.
<Primary antibody>Rabbit polyclonal to CD133-Stem Cell Marker (Abcam)
The antibody was diluted 1:35 with 1%BSA/PBS <conventional>
or *Can Get Signal*® immunostain Solution A or B
<Secondary antibody> Envision (Dako ChemMate)
<Detection> DAB (Dako)

* The data was kindly provided by Dr.Nodagashira and Dr. Sasai , Graduate School of Medicine, Hokkaido University

Applications

- Immunohistochemistry
- Immunocytochemistry

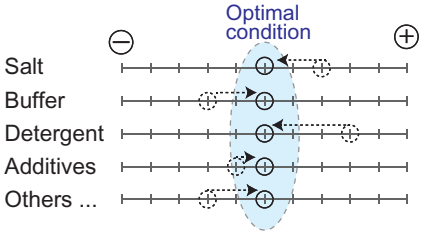


Flow chart of ABC staining with *Can Get Signal*® immunostain

Principle

Our “immunoreaction enhancing technology (IE technology)” is based on acceleration of antigen-antibody reaction whilst keeping high specificity and low background by optimization of the antibody diluents. This technology is applied to the *Can Get Signal*® (Code No. NKB-101) and *Can Get Signal*® immunostain (Code No. NKB-401) series.

Can Get Signal® immunostain (Code No. NKB-401) is a reaction solution that contains an accelerator for antigen-antibody reactions, which improves sensitivity, specificity, and S/N of immunohistochemistry (IHC) and immunocytochemistry.



Application data

1. Detection of PCNA using paraffin-embedded sections

The localization of PCNA (proliferating cell nuclear antigen) expression in human skin was detected using paraffin-embedded sections of the human skin tissue model TESTSKIN™ (Toyobo). Detection was performed by the ABC method with anti-PCNA mouse monoclonal antibody as the primary antibody and biotinylated mouse IgG as the secondary antibody. Each antibody was diluted with Solution A of *Can Get Signal*® immunostain prior to use. As a control experiment, PBS(-) containing 1.5% normal horse serum (conventional method) was used instead of Solution A of *Can Get Signal*® immunostain. As a result, *Can Get Signal*® immunostain produced higher signals and lower background than the conventional method.

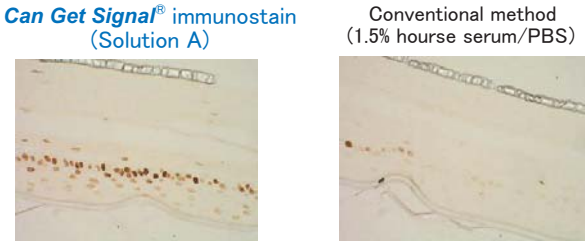


Fig. IHC detection of PCNA using paraffin-embedded tissue sections

2. Detection of paxillin with the fluorescent antibody method

The localization of paxillin in Swiss 3T3 cells was detected using anti-paxillin polyclonal antibody and Alexa488-conjugated rabbit IgG antibody. As a result, the exposure time for detection could be reduced from 3 s to 1 s by using *Can Get Signal*® immunostain, and the detailed fiber structure (fibrillar adhesion) could also be detected by using *Can Get Signal*® immunostain. However, the structure could not be detected with the conventional method due to high background signals.

Fig. C shows a merged picture of the immunological detection with anti-paxillin and anti-phospho-tyrosine antibodies, and actin staining.

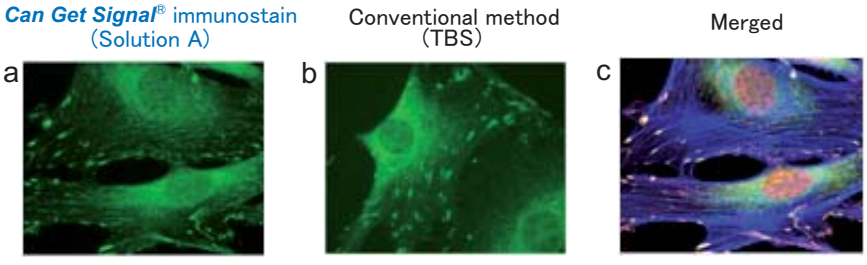


Fig. Immunocytochemistry detection of paxillin with the fluorescent antibody method
* The data was kindly provided by Dr. Harada, Tokyo Institute of Technology.

Hybridization accelerator

PerfectHyb® hybridization solution

Code No. HYB-101 250 ml

Store at room temperature

Components:

Perfecthyb® hybridization solution	250 ml

PerfectHyb® hybridization solution contains an accelerator that improves the hybridization efficiency and background. The hybridization rate is accelerated and background hybridization minimized. The solution facilitates Southern and northern blot analyses.

Features

- **Enables efficient and sensitive RI and non-RI detections on Southern and northern blot analyses.**
- **Reduces hybridization time from 16 to 1-2 hr.**
- **Over night hybridization significantly increases sensitivity for the detection of low abundance mRNAs by northern blot analyses.**
- **Low viscosity and no generation of precipitates at room temperature.**
- **Addition of salmon sperm DNA is not necessary.**
- **Hybridization and washing steps can be performed at the same temperature.**

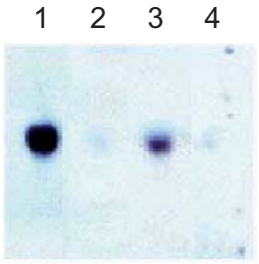
Applications

- Southern blot
- Northern blot

Application data

1. Comparison of efficiency for northern blot analysis

The transferrin receptor mRNA was detected efficiently with low background on Northern blot analysis using PerfectHyb®.

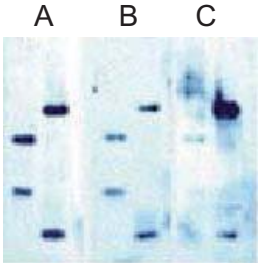


Sample: HeLa cell total RNA 5μg.
Target: Transferrin receptor gene
Probe: [^{32}P]-labeled cDNA probe prepared by the random priming method.

1: PerfectHyb®, 2hr
2: Company A, 2hr
3: Company B, 2hr
4: Conventional method, 16hr

2. Comparison of the efficiency and specificity for detecting a gene polymorphism by Southern blot

Polymorphic bands were efficiently detected with low background on Southern blot analysis using PerfectHyb®.



Sample: Genomic DNA *Hae* III digested 1 μg.
Target: YNH24(D2S44)
Probe: [^{32}P]-labeled probe

A: PerfectHyb®, 2hr
B: Company A, 2hr
C: Company B, 2hr

ISO 9001 Certified

The quality management system of Tsuruga Biochemical plant and Tsuruga Institute of Biotechnology of Toyobo Co.,Ltd. have been approved in UK, USA, Holland, Germany and Australia as well as in Japan and meet the requirements of ISO 9001 according to Lloyd' s Resister Quality Assurance Limited (LRQA).
Among the products listed here, modifying enzymes manufactured in this factory are managed and maintained in accordance with the quality management system.

ISO 14001 Certified

Tsuruga Plant of Toyobo Co.,Ltd. is ISO14001 certified by JIC Quality Assurance Ltd. (JICQA) , a certification / registration body accredited by the Japan Accreditation Board for Conformity Assessment (JAB), an accreditation body for environmental management systems.
In Tsuruga Biochemical Operations Dept. of Toyobo Co., Ltd., we are also proceeding with R&D and manufacturing activity to apply an environment management system to the Tsuruga Plant white biotechnology related products manufactured here are developed and manufactured in our ISO 1400 certified factory / laboratory.

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