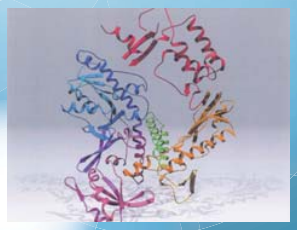
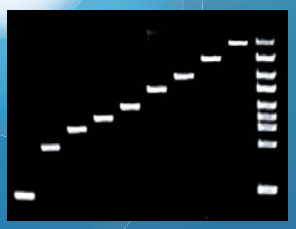
TOYOBO
Ideas & Chemistry





TOYOBO
Life Science

Raw Material Catalog

- Real-time PCR Master Mix
- Raw Materials
- PCR Kits
- NGS Related Products

C O N T E N T S

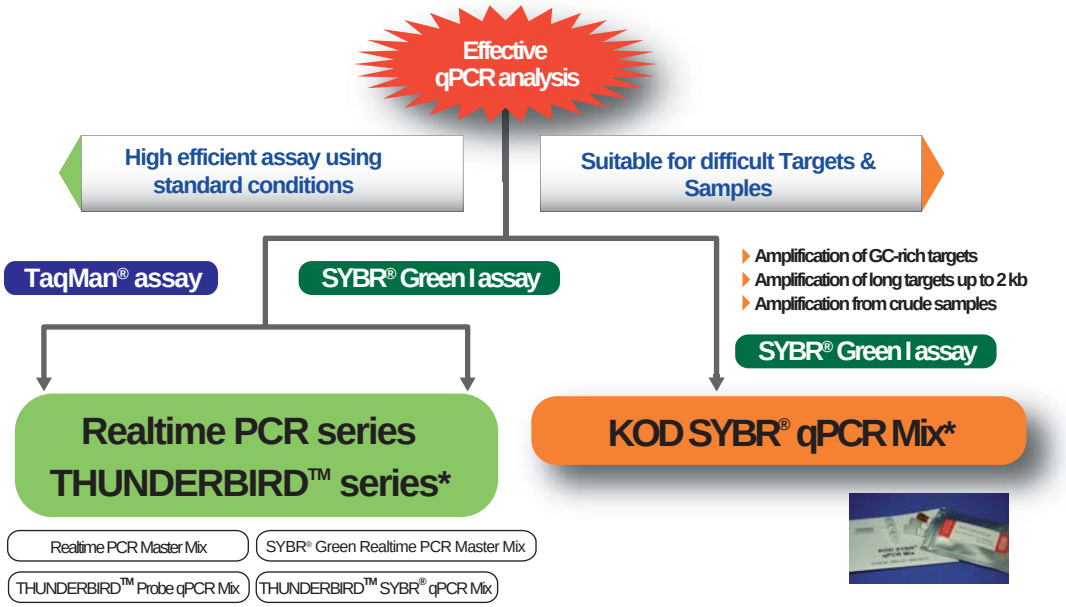
Real-time PCR Master Mix selection guide

TOYOBO has various realtime PCR master mixes from which to choose. The following table shows the characteristics of each product. The Realtime PCR Master Mix series and the THUNDERBIRD™ qPCR Mix series are Taq DNA polymerase-based 2x master mixes for rea-ltime 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 enable appropriate application depending on the realtime cycler used. The *RNA-direct*™ series is a 2x master mix for one-step realtime RT-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 one-step realtime RT-PCR, including both reverse transcription and PCR steps. THUNDERBIRD™ Probe One-step qRT-PCR Kit was developed with a focus on the sensitivity as a one-step real-time RT-PCR kit using the highly efficient reverse transcriptase "ReverTra Ace™" and Tth DNA Polymerase as a PCR enzyme.
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 (<2 kb) and GC-rich targets. It is also applicable to amplification from crude samples, such as whole blood, microorganisms, and various lysates from animal and plant tissues.

Template	Enzyme	Product Name	Assay type	One-step RT-PCR	Hot start (Antibody)	Passive reference	Sensitivity	Specificity	Efficiency	Long target amplification	GC-rich targets	Amplification from crude samples	Reference page
DNA	rTaq DNA polymerase	Realtime PCR Master Mix	Probe		✓	✓	+++	++	+++	++	+	+	2
		SYBR® Green Realtime PCR Master Mix	SYBR®		✓	✓	+++	++	+++	++	+	+	
		THUNDERBIRD™ Probe qPCR Mix	Probe		✓	✓*	+++	+++	++	+	+	+	4
		THUNDERBIRD™ SYBR® qPCR Mix	SYBR®		✓	✓*	+++	+++	++	+	+	+	
	KOD exo(-) DNA polymerase	KOD SYBR® qPCR Mix	SYBR®		✓	✓*	+++	+++	+++	+++ (<2kb)	+++	+++	6
RNA	rTth DNA polymerase	*RNA-direct*™ Realtime PCR Master Mix	Probe	✓ 1enzyme	✓	✓	++	++	++	++	++	++	10
		RNA-direct™ SYBR® Green Realtime PCR Master Mix	SYBR®	✓ 1enzyme	✓	✓	++	++	++	++	++	++	
		THUNDERBIRD™ Probe One-step qRT-PCR Kit	Probe	✓ 2enzymes**	✓	✓*	+++	+++	+++	++	++	+++	8

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

*50x ROX reference dye is individually supplied.
**This product contains ReverTra Ace™ and Tth DNA Polymerase.



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

Realtime PCR Master Mix Series

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.

This product can be applied to one-step RT-PCR by adding reverse transcriptase (ReverTra Ace™).

Store at -20 °C

Components:

<Probe Version>

Realtime PCR Master Mix*

5 x 1 ml (Code No. QPK-101)
50 ml (Code No. QPK-119CH)
100 ml (Code No. QPK-159)

<SYBR® Green Version>

SYBR® Green Realtime PCR Master Mix*

5 x 1 ml (Code No. QPK-201)
100 ml (Code No. QPK-259)

*The reagents contain 2x ROX reference dye.

Features

- **High specificity and effective amplification enable the detection of low-copy targets.**

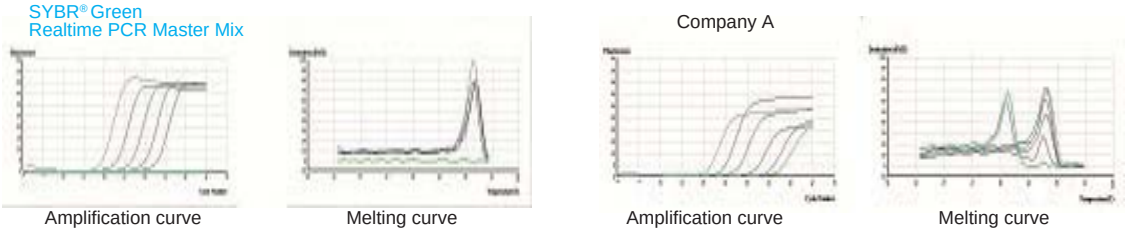
Table Compatible real-time PCR instruments

Applied Biosystems	ABI PRISM® 7000 ABI PRISM® 7700 Applied Biosystems® 7300 Applied Biosystems® 7900HT Applied Biosystems® StepOne™ Applied Biosystems® StepOne Plus™	**Roche Diagnostics**	LightCycler® 1.x / 2.0 LightCycler® Nano LightCycler® 480 / LightCycler® 96
		Bio-Rad/MJ	MiniOpticon™/ CFX96 Touch™
		TaKaRa	Thermal Cycler Dice® Real Time System
		QIAGEN	Rotor-Gene

Application data

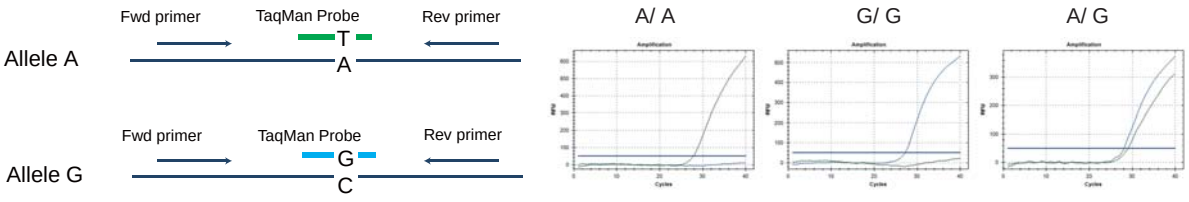
1. Detection of β-actin gene

Amplification of the β-actin gene was carried out using serially diluted genomic DNA solutions (10^n dilutions; 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.



2. Detection of CYP2C19*2 SNP

A single nucleotide polymorphism (SNP) of CYP2C19*2 detected using human genomic DNA with allele specific TaqMan® probes and Realtime PCR Master Mix [Code No. QPK-101] .

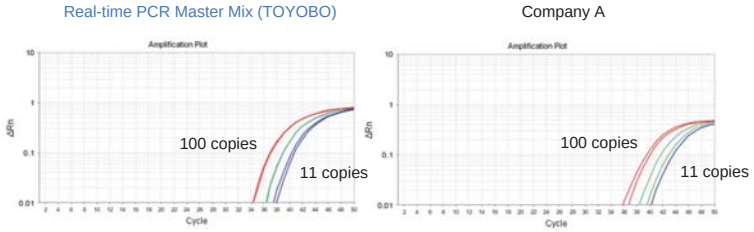


Green: A specific probe

Blue: G specific probe

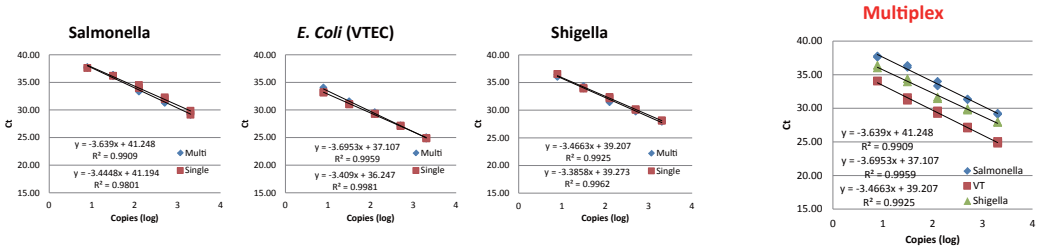
3. Detection of papilloma virus (HPV) DNA

The sensitivity and quantitativity of two master mix reagents were compared by detecting serially (3^n) diluted HPV DNA (100, 33, 11 copies) using TaqMan® probe. Real-time PCR Master Mix (TOYOBO) exhibited higher sensitivity and PCR efficiency.



4. Detection of Enteric bacteria DNA

The target genes were detected from serially (4^n) diluted purified genomic DNA of Salmonella, Escherichia coli (VTEC) and Shigella cells by triplex detection systems with TaqMan® probes labeled by different fluorescent dyes. No significant differences of PCR efficiency and correlation coefficient were observed between the triplex and singleplex systems.



Related products

Realtime PCR Master Mix (Code No. QPK-101) is standard type of master mix based on Taq DNA Polymerase. It consists of various components such as enzymes, buffers, dNTPs etc. TOYOBO supplies basic and optional components so that researchers can modify the reagents depending on the situation. By using the standard option, the product can be reconstituted.Some of the components (enzymes or buffers) contain glycerol which inhibit lyophilization. TOYOBO supply glycerol-free components.

	Product name	Options						Reference page
		Standard	Without ROX dye	carry-over prevention	Glycerol free	carry-over prevention & Glycerol free	One-step RT-PCR	
Basic component	rTaq DNA Polymerase Hot Start	✓	✓	✓			✓	12
	10x Buffer for Taq Hot Start	✓	✓	✓	✓	✓	✓	13
	dNTPs	✓	✓	✓	✓	✓	✓	20
	50x ROX reference dye	✓		✓	✓	✓	✓	17
Optional component	Uracil-DNA-Glycosylase			✓				19
	dUTP			✓		✓		20
	ReverTra Ace™						✓	20
Glycerol free component	rTaq DNA Polymerase Hot Start <Glycerol Free>				✓	✓		12
	Uracil-DNA-Glycosylase <Glycerol Free>					✓		19

THUNDERBIRD™ qPCR Mix Series

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 is enhanced.

Store at -20 °C

Components:

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

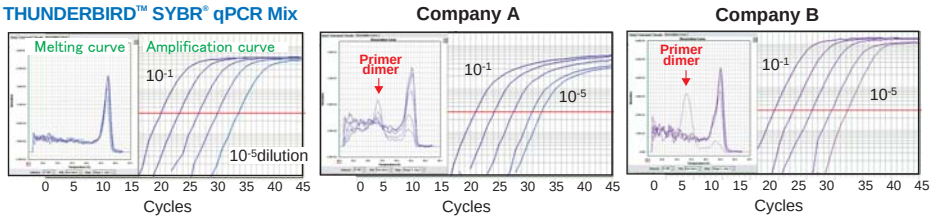
THUNDERBIR™ Probe qPCR Mix	3 x 1.67 ml
50x ROX reference dye	250 µl

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

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

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 gene 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

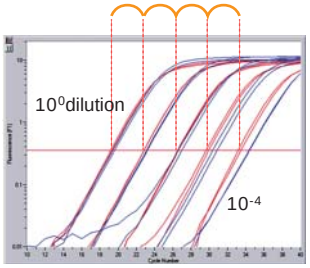
Table Compatible real-time PCR instruments

Applied Biosystems	ABI PRISM® 7000 ABI PRISM® 7700 Applied Biosystems® 7300 Applied Biosystems® 7500/7500Fast Applied Biosystems® 7900HT Applied Biosystems® StepOne™ Applied Biosystems® StepOne Plus™ ViiA™ 7 QuantStudio®	**Roche Diagnostics**	LightCycler® 1.x / 2.0 LightCycler® Nano LightCycler® 480 / LightCycler® 96
		Bio-Rad/MJ	MiniOpticon™/ CFX96 Touch™
		Agilent Technologies	Mx3000P/ Mx3005P/ Mx4000
		TaKaRa	Thermal Cycler Dice® Real Time System
		QIAGEN	Rotor-Gene

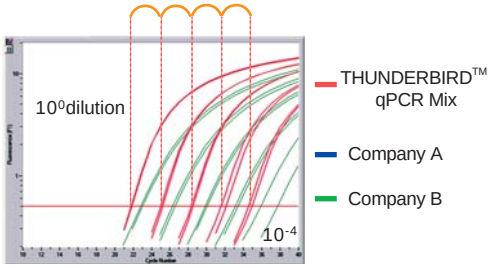
Application data

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® realtime PCR. THUNDERBIRD™ qPCR Master Mix showed greater efficiency than other reagents.



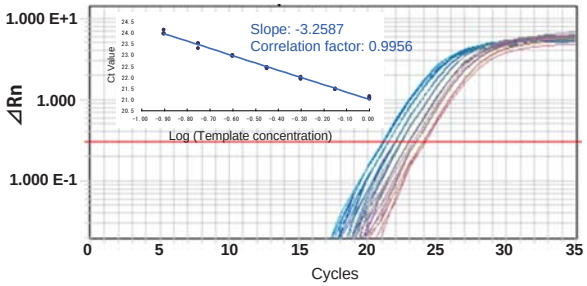
Detection of Norovirus G1 gene
by SYBR® Green I assay.
[Roche Diagnostics LightCycler 1.1]



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 2.5 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

Store at -20 °C
Components:
Code No. QKD-201
200 reactions [50 μl per reaction]

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

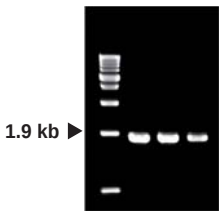
KOD SYBR® qPCR Mix is a highly efficient 2x master mix for real-time PCR using SYBR® Green I and the 3'→5' exonuclease deficient KOD DNA Polymerase. The master mix contains all of the required components, except for the primers and the ROX reference dye (50x ROX reference dye is supplied separately with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

Features

- **Quantitative amplification for long targets(~ 2kb).**



Same primers

Target: TGFβ (1,918 bp)
Template: genomic DNA
M: 1 kb Ladder

<Electrophoresis analysis>

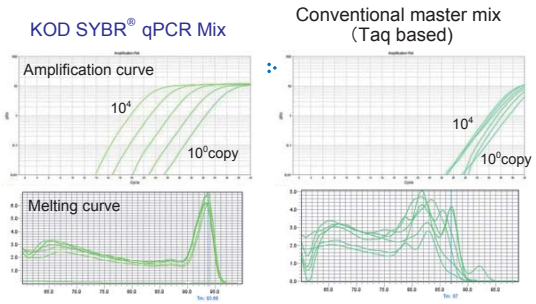
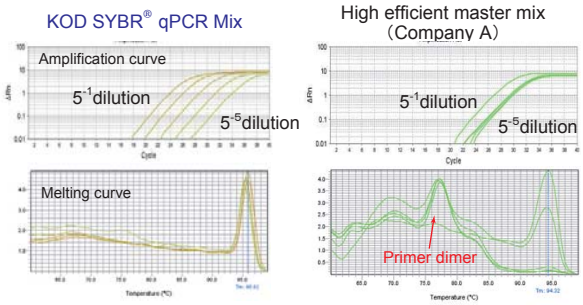


Fig. Long target amplification [ABI StepOnePlus™]

- **Efficient amplification 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™]

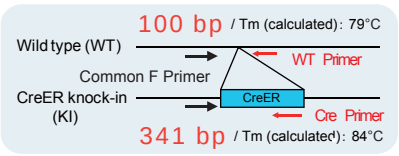
- **Effective amplification from crude samples.**

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 26)
plant tissue	lysate (p 26)

● **Genotyping of knock-in mice using 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 26)
Primer ratio: F: WT: Cre = 0.2: 0.2: 0.67 mM (final)
Sample: Mouse tail lysate 2 μl / 20 μl reaction

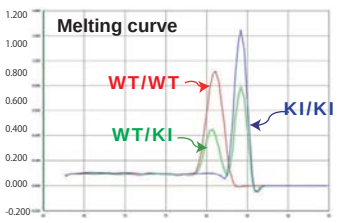


Fig. One-tube mouse genotyping using melting curve analysis [ABI 7500 Fast]

High efficient real-time PCR Master Mix

Applications

- Real-time PCR

Table　Compatible real-time PCR 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™ ViiA7™ QuantaStudio®	**Roche Diagnostics**	LightCycler® 1.x / 2.0 LightCycler® Nano LightCycler® 480 / LightCycler® 96
		Bio-Rad/MJ	MiniOpticon™/ CFX96 Touch™
		Agilent Technologies	Mx3000P/ Mx3005P/ Mx4000
		TaKaRa	Thermal Cycler Dice® Real Time System
		QIAGEN	Rotor-Gene

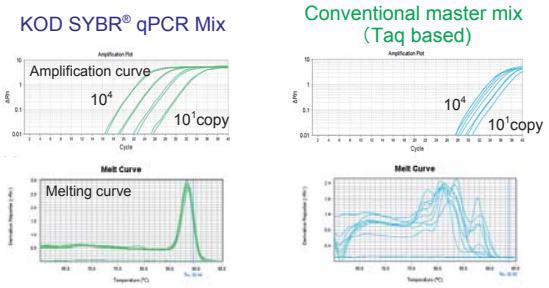
Application data

1. Comparison of the PCR efficiency on a ChIP target

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.

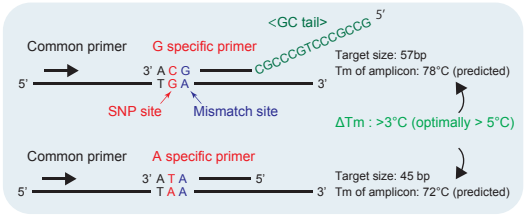
CCCGCCCGAGAGAGTGACTCTCACGAGAGCCGCGAGAGTCAGCT
TGGCCAATCCGTGCGGTCGGCGGCCGCTCCCTTTATAAGCCGACT
CGCCCGGCAGCGCACCGGGTTGCGGAGGGTGGGCCTGGGAGGG
GTGGTGGCCATTTTTTGTCTAACCCTAACTGAGAAGGGCGTAGGC
GCCGTGCTTTTGCTCCCCGCGC**GCTGTTTTTCTCGCTGACTT**

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



2. SNP analysis using whole blood samples

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).



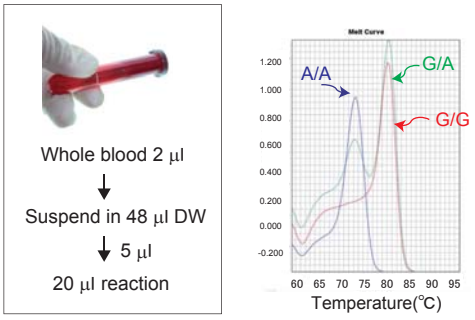


Fig.　One-tube ASP-PCR analysis using whole blood specimen. [ABI 7500 Fast real-time PCR system]

THUNDERBIRD™ Probe One-step qRT-PCR Kit

THUNDERBIRD™ Probe One-step qRT-PCR Kit is a one-step realtime reverse-transcription polymerase chain reaction (RT-PCR) kit using the highly efficient reverse transcriptase "ReverTra Ace™" and Tth DNA polymerase as a PCR enzyme. This product can be used mainly in TaqMan® probe assays. The one-step system is suitable for high-throughput analysis because of its simple reaction setup. In addition, this system can reduce the risk of cross-contamination. The combination of the two enzymes and optimized buffer system enable the effective detection and quantification of a small amount of RNA. This kit can also detect various kinds of RNA with different sequences because it is tolerant of target sequence diversity.

Store at -20 °C
Components:

Code No. QRZ-101

100 reactions [50 µl per reaction]

2x Reaction Buffer*	2 × 1.25 ml
DNA Polymerase	125 µl
RT Enzyme Mix	125 µl
50x ROX reference dye	100 µl
RNase free water	2 × 1.25 ml

Code No. QRZ-129B

10,000 reactions [50 µl per reaction]

2x Reaction Buffer*	250 ml
DNA Polymerase	12.5 ml
RT Enzyme Mix	12.5 ml

* 2x Reaction Buffer contains essential components for the reaction (buffer, Mg^{2+}, salts, dATP, dCTP, dGTP, and dUTP, etc.).

Features

- **Rapid and highly sensitive detection**
- **Tolerant of target sequence diversity**
- **Utilization of dUTP**

  This kit contains dUTP instead of dTTP in 2× Reaction Buffer. Therefore, the rate of false-positive detection can be reduced by adding uracil-N-glycosylase (UNG).
  *UNG is not supplied with this kit.

- **Tolerant of PCR inhibitors**

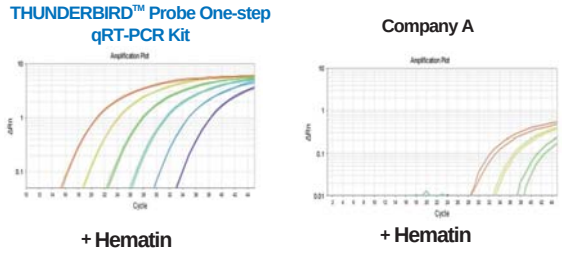


Fig The effect of inhibition by hematin
The addition of 2 mM hematin did not affect the reaction.

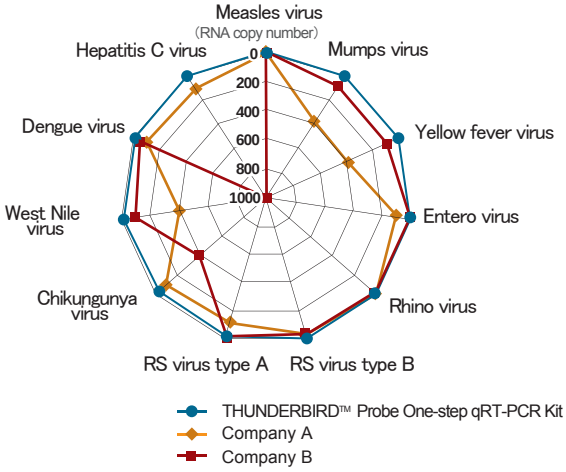


Fig The maximum sensitivities of various one-step qRT-PCR kits
A 4^n dilution series of various viral RNAs was detected. The primers and TaqMan® probes were synthesized in accordance with previous reports. The graph indicates the minimum copy numbers that were detected by the kits. THUNDERBIRD™ Probe One-step qRT-PCR Kit was the only kit that detected all viral RNAs tested at high sensitivity (≤30 copies).

Applications

- Realtime qRT-PCR

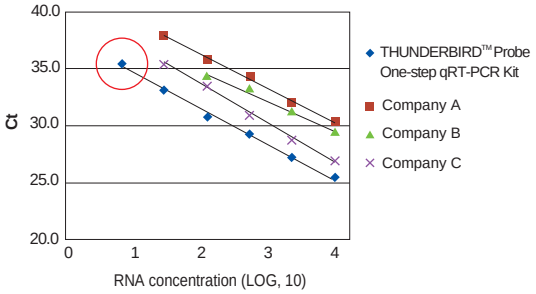
Table Compatible real-time PCR instruments

Applied Biosystems	ABI PRISM® 7000 ABI PRISM® 7700 Applied Biosystems® 7300 Applied Biosystems® 7500/7500Fast Applied Biosystems® 7900HT Applied Biosystems® StepOne™ Applied Biosystems® StepOnePlus™ ViiATM QuantaStudio®	**Roche Diagnostics**	LightCycler® 1.x / 2.0 LightCycler® Nano LightCycler® 480 / LightCycler® 96
		Bio-Rad/MJ	MiniOpticon™/ CFX96 Touch™
		Agilent Technologies	Mx3000P/ Mx3005P/ Mx4000
		TaKaRa	Thermal Cycler Dice® Real Time System
		QIAGEN	Rotor-Gene

Application data

1. Comparison of sensitivity of detection of enterovirus RNA

The sensitivity and quantitativity of various kits were compared by detecting serially (4^n) diluted enterovirus RNA . The primers and probe were synthesized in accordance with a previous report. Applied Biosystems® StepOnePlus™ was used in this experiment. THUNDERBIRD™ Probe One-step qRT-PCR Kit was the only kit that detected less than 10 copies of RNA and showed wide-ranging quantitation. The results indicate that this kit is suitable for the highly sensitive detection of RNA viruses or mRNA expressed at a low level.



2. Comparison of maximum sensitivity of various kits

The expression levels of IL-1b, TNF-a and GAPDH mRNAs were analyzed using 10^n times serially diluted total RNA (1 pg–100 ng) by triplex detection systems with TaqMan® probes labeled by different fluorescent dyes (Fig.1). LightCycler® 96 (Roche Diagnostics) was used in this experiment. HeLa S3 cells were incubated for 20 h after being seeded in six-well plates at 4×10^5 cells/well and treated with or without 100 nM phorbol 12-myristate 13-acetate. Then, the expression levels of mRNA were analyzed using purified total RNA from treated cells. The elevations of IL-1b and TNF-a mRNAs were observed upon adding phorbol 12-myristate 13-acetate (Fig. 2).
No significant differences of PCR efficiency and correlation coefficient were observed between the triplex and singleplex systems (data not shown).

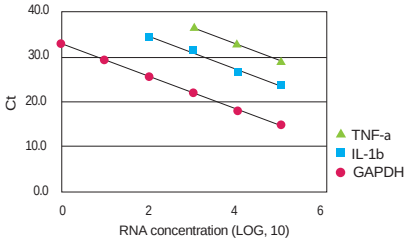


Fig. 1 Multiplex assay results

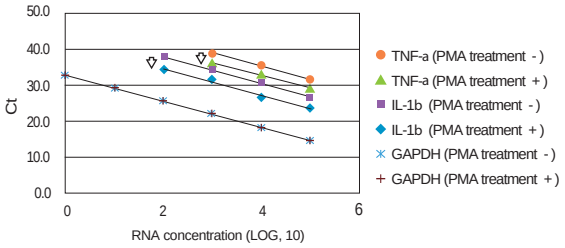


Fig. 2 Expression analysis by multiplex assay

3. Simultaneous quantitation of RS virus types A and B from clinical specimens

RT-PCR and qRT-PCR analyses of RS virus were performed using RNA purified from 20 throat swab specimens. In qRT-PCR with THUNDERBIRD™ Probe One-step qRT-PCR Kit, duplex assay was performed using specific primers and TaqMan® probes for types A and B in a single tube. LightCycler® 96 (Roche Diagnostics) was used in this experiment. In parallel, an antibody test was performed using the same swab samples.
The detection of the virus using the THUNDERBIRD™ Probe One-step qRT-PCR Kit showed a high correlation with that by RT-PCR and successfully gave quantitative values.

Table Results of correlation tests on RS virus detection by three methods.

Specimen number	Antibody test	RT-PCR (Typing)	Quantitative value (Estimated RNA copy number)	
			Type A (FAM, 470nm)	Type B (Cy5, 645nm)
#1	+	+ (A)	1.6×10^5	-
#2	-	-	-	-
#3	+	+ (A)	8.1×10^4	-
#4	+	+ (A)	2.7×10^5	-
#5	-	+ (A)	9.6×10^2	-
#6	-	+ (A)	3.6×10^3	-
#7	+	+ (A)	2.3×10^6	-
#8	-	-	-	-
#9	+	+ (A)	1.5×10^5	-
#10	+	+ (A)	6.7×10^5	-
#11	-	-	-	-
#12	+	+ (A)	1.6×10^5	-
#13	+	+ (A)	9.4×10^3	-
#14	-	+ (A)	9.5×10^3	-
#15	+	+ (A)	3.9×10^3	-
#16	+	+ (A)	2.4×10^5	-
#17	-	-	-	-
#18	-	+ (A)	4.2×10^4	-
#19	-	+ (A)	3.4×10^2	-
#20	-	+ (B)	-	9.6×10^3

RNA-direct™ Realtime PCR Master Mix Series

RNA-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 [p16]. 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.**

Applications

One-step real-time PCR

Store at -20 °C

Components:

<Probe Version>

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

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

Code No. QRT-159

RNA-direct™ Realtime PCR Master Mix*	100 ml

Code No. QRT-MN1

50 mM $Mn(OAc)_2$	20 ml

<SYBR® Green Version>

Code No. QRT-201
100 reactions [50 µl per reaction]

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

Code No. QRT-259

RNA-direct™ SYBR® Green Realtime PCR Master Mix*	100 ml

Code No. QRT-MN1

50 mM $Mn(OAc)_2$	20 ml

*The reagents contain 2x ROX reference dye.

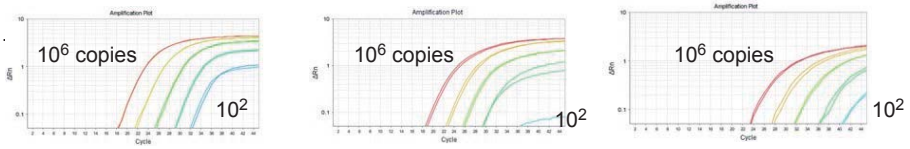
Table Compatible real-time PCR instruments

Applied Biosystems	ABI PRISM® 7000 ABI PRISM® 7700 Applied Biosystems® 7300 Applied Biosystems® 7900HT Applied Biosystems® StepOne™ Applied Biosystems® StepOne Plus™	**Roche Diagnostics**	LightCycler® 1.x / 2.0 LightCycler® Nano LightCycler® 480 / LightCycler® 96
		Bio-Rad/MJ	MiniOpticon™/ CFX96 Touch™
		TaKaRa	Thermal Cycler Dice® Real Time System
		QIAGEN	Rotor-Gene

Application data

1. Detection of hepatitis C virus (HCV)

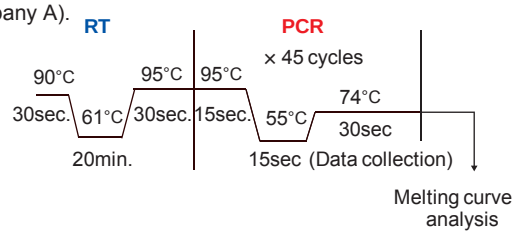
The sensitivity and quantitativity of three one-step qRT-PCR master mix reagents were compared by detecting serially (10^n) diluted HCV RNA using TaqMan® probe. *RNA-direct*™ Real time PCR Master Mix (TOYOBO) exhibited higher sensitivity and PCR efficiency than other master mixes.



RNA-direct™ Company A Company B

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).



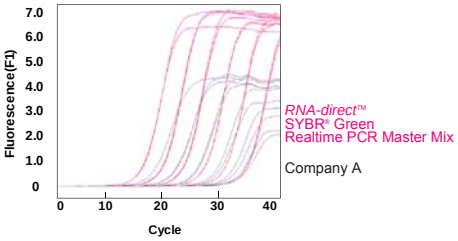
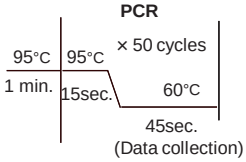


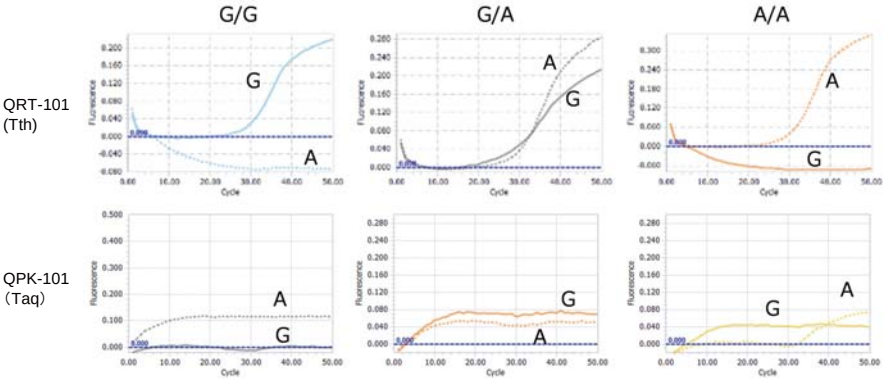
Fig. Comparison of the SYBR® Green assay

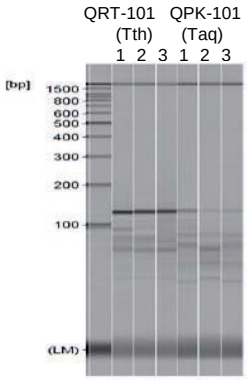
3. Detection of single nucleotide polymorphism (SNP) using fresh whole blood

The SNPs of aldehyde dehydrogenase 2 gene (ALDH2) were analyzed using fresh whole blood samples by *RNA-direct*™ Realtime PCR Master Mix (QRT-101) and Realtime PCR Master Mix (QPK-101) based on Tth and Taq DNA polymerase, respectively. QRT-101 successfully detected all types of SNPs (G/G, G/A, A/A) using TaqMan® probe assay. In this experiment, the amplification by QRT-101 was performed in the presence of $MgCl_2$ instead of $Mn(OAc)_2$.

DW	5.4
RNA-direct™ Realtime PCR Master Mix	10
25mM $MgCl_2$	2
10uM Fwd	0.4
10uM Rev	0.4
10uM Pb1	0.4
10uM Pb2	0.4
Whole blood	1
	20 µl







Related products

RNA-direct™ Realtime PCR Master Mix (Code No. QRT-101) consists of various components such as enzymes, buffers, and dNTPs, etc. TOYOBO supplies components individually contained in *RNA-direct*™ Realtime PCR Master Mix (QRT-101). Researchers can modify the RT-PCR reaction mixture depending on the situation. By using the standard option, the products (QRT-101) can be reconstituted.Some of the components (enzymes or buffers) contain glycerol which inhibit lyophilization. TOYOBO supply glycerol-free components.

	Product name	Options								Reference page
		Tth DNA Polymerase			TTx DNA Polymerase*					
		Standard	Without ROX dye	carry-over prevention	Standard	Without ROX dye	carry-over prevention	Glycerol free	carry-over prevention & Glycerol free	
Basic component	Hot Start rTth DNA Polymerase	✓	✓	✓						15
	5x Buffer for rTth/ TTx (DNA/RNA)	✓	✓	✓	✓	✓	✓			16
	50mM $Mn(OAc)_2$	✓	✓	✓	✓	✓	✓	✓	✓	17
	dNTPs	✓	✓	✓	✓	✓	✓	✓	✓	20
	50x ROX reference dye	✓		✓	✓		✓	✓	✓	17
Optional component	Uracil-DNA-Glycosylase			✓			✓			19
	dUTP			✓			✓		✓	20
	Hot Start TTx DNA Polymerase				✓	✓	✓			15
Glycerol free component	Hot Start TTx DNA Polymerase <Glycerol free>							✓	✓	15
	5x Buffer for rTth/ TTx (DNA/RNA) <Glycerol free>							✓	✓	16
	Uracil-DNA-Glycosylase <Glycerol free>								✓	19

*TTx DNA Polymerase exhibits higher PCR efficiency than Tth DNA Polymerase. The polymerase shows the reverse transcriptase activity in the presence of Mn^{2+} like Tth DNA Polymerase. The polymerase enables highly sensitive and fast detection from small amount of RNA with one-step qRT-PCR and relatively tolerant of typical PCR inhibitors.

rTaq DNA Polymerase Hot Start

Coming soon

rTaq DNA Polymerase Hot Start <Glycerol Free>

rTaq DNA Polymerase Hot Start is a mixture of recombinant Taq DNA Polymerase derived from *Thermus aquaticus* YT-1 and anti-Taq DNA Polymerase antibodies for hot start PCR (anti-Taq high [Code No. TCP-101]). The hot start antibodies can reduce the non-specific amplification due to preventing mis-priming. The antibodies are inactivated at the initial denaturation step and do not inhibit subsequent steps.

rTaq DNA Polymerase Hot Start <Glycerol Free> is glycerol-free type of rTaq DNA Polymerase Hot Start . The reagent can be used in a preparation of master mix reagents for lyophilization.

Store at -20 °C

Components:

Code No. TAP-329E 10,000 U	
rTaq DNA Polymerase Hot Start (5 U/μl)	2 ml
Code No. TAP-359E 100,000 U	
rTaq DNA Polymerase Hot Start (5 U/μl)	20 ml
Code No. TAP-329GF 10,000 U	
rTaq DNA Polymerase Hot Start <Glycerol Free>	2 ml
Code No. TAP-359GF 100,000 U	
rTaq DNA Polymerase Hot Start <Glycerol Free>	20 ml

Features

- **The polymerase can be reactivated quickly compared with a chemically modified polymerase**
- **The polymerase can be used in the reconstitution of Realtime PCR Master Mix (Code No. QPK-101).**

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.

Store at -20 °C

Components:

Code No. TAP-201 250 U	
<100~200 reactions [50 μl per reaction]>	
rTaq DNA Polymerase (5 U/μl)	50 μl
10× PCR Buffer*	1 ml
25 mM $MgCl_2$	1 ml
2 mM dNTPs	1 ml
Code No. TAP-229E 10,000 U	
rTaq DNA Polymerase (5 U/μl)	2 ml
Code No. TAP-259E 100,000 U	
rTaq DNA Polymerase (5 U/μl)	20 ml
Code No. TAP-279E 1,000,000 U	
rTaq DNA Polymerase (5 U/μl)	200 ml

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

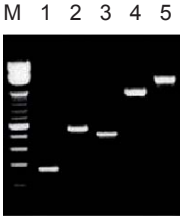
Features

- **Tolerates various kinds of PCR protocols.**
- **Applicable for hot start technology by adding anti-Taq antibody "anti-Taq high " (Code No. TCP-101) [p18].**
- **PCR products can be cloned by using a TA cloning method.**
- **Applicable for PCR using dUTP, dITP and fluorescently-labled nucleotide.**

Applications

- PCR
- Primer extension

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

	X (μl)
DW	
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
	20

94°C, 2min.
94°C, 10sec.
(Tm-5)°C, 30sec.
72°C, 1min./ kb
25~40 cycles

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)

10x Buffer for Taq Hot Start

Coming soon

10 x Buffer for Taq Hot Start is a concentrated buffer components, which consists of buffer, Mg^{2+} and salts, used in Realtime PCR Master Mix (QPK-101). By mixing with rTaq DNA Polymerase Hot Start, dNTPs and ROX reference dye, QPK-101 can be reconstituted. The buffer does not contain glycerol which inhibits lyophilization.

Store at -20 °C

Components:

Code No. QPK-1B1

10x Buffer for Taq Hot Start * 20 ml

Code No. QPK-1B2

10x Buffer for Taq Hot Start * 200 ml

* 10x Buffer doesn' t contain dNTPs and enzyme.

Features

- **The buffer can be used in the reconstitution of Realtime PCR Master Mix (Code No. QPK-101).**

Raw materials can be applied to Realtime PCR. Using these raw materials related Taq DNA Polymerase, Realtime PCR Master Mix (Code No. QPK-101) can be reconstituted. TOYOBO supplies basic and optional components so that researchers can modify the reagents depending on the situation. Some of the components (enzymes or buffers) contain glycerol which inhibit lyophilization. TOYOBO supply glycerol-free components.

	Product name	Options						Reference page
		Standard	Without ROX dye	carry-over prevention	Glycerol free	carry-over prevention & Glycerol free	One-step RT-PCR	
Basic component	rTaq DNA Polymerase Hot Start	✓	✓	✓				12
	10x Buffer for Taq Hot Start	✓	✓	✓	✓	✓	✓	13
	dNTPs	✓	✓	✓	✓	✓	✓	20
	50x ROX reference dye	✓		✓	✓	✓	✓	17
Optional component	Uracil-DNA-Glycosylase			✓				19
	dUTP			✓		✓		20
	ReverTra Ace™						✓	20
Glycerol free component	rTaq DNA Polymerase Hot Start <Glycerol Free>				✓	✓		12
	Uracil-DNA-Glycosylase <Glycerol Free>							19

Raw material related Tth/ TTx DNA Polymerase

Tth DNA Polymerase and TTx DNA Polymerase relatively tolerant of typical PCR inhibitor. Tth/ TTx are useful to amplify DNA from crude samples. And these enzymes exhibit reverse transcriptase activity in the presence of Mn^{2+} ions. These enzymes can be applied to one-step RT-PCR, including reverse transcription and PCR steps. These polymerase exhibits double strand specific 5' →3' exonuclease, can be applied to TaqMan® probe assay.

Tth DNA Polymerase and TTx DNA Polymerase have higher efficiency than rTaq DNA Polymerase. And these enzymes relatively tolerant of typical PCR inhibitor.
Please use the following combinations for DNA amplification.

Amplification of DNA		Enzyme	Specificity	Efficiency	Amplificaton from crude sample	lyophilization	Economy	Reference page
Reaction Buffer	2x Buffer for Tth/ TTx (DNA) (containing dNTP, dUTP, Mg^{2+})	Hot Start rTth DNA Polymerase	+++	+++	+++		+	15
		Hot Start TTx DNA Polymerase	+++	++++	++++		+	15
	5x Buffer for Tth/ TTx (DNA/RNA) dNTPs 25 mM $MgCl_2$	Hot Start TTx DNA Polymerase<Glycerol Free>	++	++	++	✓	+++	15
Conventional Taq DNA polymerase reaction			++	++	+		++	-
Optional component		50x ROX reference dye						17
		Uracil-DNA-Glycosylase						19
		Uracil-DNA-Glycosylase <Glycerol Free>						19
		dUTP						20

Tth/ TTx DNA Polymerase enable 1 enzyme one-step RT-PCR in the presence of Mn^{2+}. Please use the following combinations for one-step RT-PCR
TOYOBO supplies basic and optional components so that researchers can modify the reagents depending on the situation. By using the standard option, *RNA-direct*™ Realtime PCR Master Mix (Code No. QRT-101, P10) can be reconstituted. Some of the components (enzymes or buffers) contain glycerol which inhibit lyophilization. TOYOBO supply glycerol-free components.

	Product name	Options								Reference page
		Tth DNA Polymerase			TTx DNA Polymerase					
		Standard	Without ROX dye	carry-over prevention	Standard	Without ROX dye	carry-over prevention	Glycerol free	carry-over prevention & Glycerol free	
Basic component	Hot Start rTth DNA Polymerase	✓	✓	✓						15
	5x Buffer for rTth/ TTx (DNA/RNA)	✓	✓	✓	✓	✓	✓			16
	50mM $Mn(OAc)_2$	✓	✓	✓	✓	✓	✓	✓	✓	17
	dNTPs	✓	✓	✓	✓	✓	✓	✓	✓	20
	50x ROX reference dye	✓		✓	✓		✓	✓	✓	17
Optional component	Uracil-DNA-Glycosylase			✓			✓			19
	dUTP			✓			✓		✓	20
	Hot Start TTx DNA Polymerase				✓	✓	✓			15
Glycerol free component	Hot Start TTx DNA Polymerase <Glycerol free>							✓	✓	15
	5x Buffer for rTth/ TTx (DNA/RNA) <Glycerol free>							✓	✓	16
	Uracil-DNA-Glycosylase <Glycerol free>								✓	19

Hot Start TTx DNA Polymerase

Coming soon

Hot Start TTx DNA Polymerase <Glycerol Free>

Hot Start TTx DNA Polymerase is a mixture of recombinant TTx DNA Polymerase derived from T*hermus sp.* and anti-Taq DNA Polymerase antibodies for hot start PCR (anti-Taq high [Code No. TCP-101]). TTx DNA Polymerase exhibits higher PCR efficiency than Tth DNA Polymerase, and relatively tolerant of typical PCR inhibitors. This product can be applied to amplfication from crude samples. And this polymerase shows the reverse transcriptase activity in the presence of Mn^{2+} like Tth DNA Polymerase. The polymerase enables highly sensitive and fast detection from small amount of RNA with one-step qRT-PCR.
The hot start antibodies can reduce the non-specific amplification due to preventing mis-priming. The antibodies are inactivated at the initial denaturation step and do not inhibit subsequent steps.
Hot Start TTx DNA Polymerase <Glycerol Free> is a glycerol -free type of Hot Start TTx DNA Polymerase. The reagent can be used in a preparation of master mix reagents for lyophilization.

Store at -20 °C
Components:

Code No. HSTTX-129 10,000 U	2.5 ml
Code No. HSTTX-159 100,000 U	25 ml
Code No. HSTTX-179 1,000,000 U	250 ml
Hot Start TTx DNA Polymerase (4 U/μl)	
Code No. HSTTX-219 1,000 U	250 μl
Code No. HSTTX-229 10,000 U	2.5 ml
Code No. HSTTX-259 100,000 U	25 ml
Hot Start TTx DNA Polymerase (4 U/μl) <Glycerol Free>	

Features

- **The polymerase can be reactivated quickly compared with a chemically modified polymerase**
- **The polymerase can be used as a component of master mix reagents.**
- **Enable one-step RT-PCR using Mn^{2+}.**
- **Relatively tolerant of typical PCR inhibitor.**

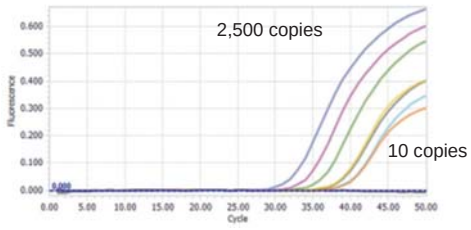
Application data

1. Detection of enterovirus RNA

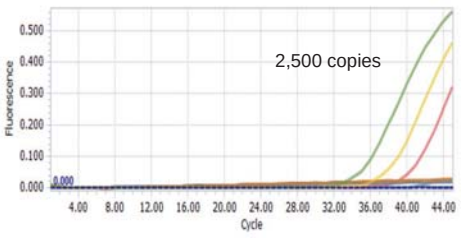
Serially (4^n) diluted Enterovirus RNAs were detected using *RNA-direct*™ Realtime PCR Master Mix based on Tth DNA Polymerase with TaqMan® probe. At the same time, the master mix based on TTx DNA Polymerase, a mixture of Hot Start rTth DNA Polymerase, 5x Buffer for Tth/TTx (DNA/RNA), dNTPs, ROX reference dye and $Mn(OAc)_2$.:[*RNA-direct*™ Real-time PCR Master Mix,TTx version].were tested using the same conditon. As a result, Tth version detected 10 copies of viral RNA.

90℃ 30sec
60℃ 5min
95℃ 1min
95℃ 3sec
60℃ 5sec
] × 50

RNA-direct™ Real-time PCR Master Mix (TTx version)



RNA-direct™ Real-time PCR Master Mix



Hot Start rTth DNA Polymerase

Coming soon

Hot Start rTth DNA Polymerase is a mixture of recombinant Tth DNA Polymerase derived from *Thermus thermophilus* HB8 and hot start antibodies. The hot start antibodies can reduce the non-specific amplification due to preventing mis-priming. The antibodies are inactivated at the initial denaturation step and do not inhibit subsequent steps.

Store at -20 °C
Components:

Code No. HSTTH-301 250 U	
Hot Start rTth DNA Polymerase (4 U/μl)	62.5 μl
2×Buffer for rTth/TTx (DNA)	1.25 ml ×2
Code No. HSTTH-329 10,000 U	
Hot Start rTth DNA Polymerase (4 U/μl)	2.5 ml

Features

- **The polymerase can be reactivated quickly compared with a chemically modified polymerase.**
- **This product can be used for the reconstitution of *RNA-direct*™ Realtime PCR Master Mix (Code No. QRT-101).**

rTth DNA Polymerase

rTth 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) and real-time PCR (Code No. QRT-101, 201, p10) using this enzyme are available.

Store at -20 °C
Components:

Code No. TTH-301 250 U
<100~200 reactions [50 μl per reaction]>

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

Code No. TTH-329L 10,000 U

rTth DNA Polymerase (5 U/μl)	2 ml

Code No. TTH-359L 100,000 U

rTth DNA Polymerase (5 U/μl)	20 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)

2x Buffer for rTth/ TTx (DNA)

Coming soon

This product is optimized buffer for Tth/ TTx DNA Polymerase, which contains Mg^{2+} and dNTP (dUTP).
This buffer provides greater efficiency and can be applied to amplification from crude sample. This buffer contains dUTP, thus allowing for carryover prevention using Uracil-DNA Glycosylase.

Features

- **High specificity and effictive amplification using Tth/ TTx DNA Polymerase.**

Store at -20 °C
Components:

Code No. QRZ-1B1	
2x Buffer for rTth/ TTx (DNA)	100 ml
Code No. QRZ-1B2	
2x Buffer for rTth/ TTx (DNA)	250 ml
Code No. QRZ-1B4	
2x Buffer for rTth/ TTx (DNA)	1000 ml

* 2x Buffer contains essential components for the reaction (buffer, Mg^{2+}, salts, dATP, dCTP, dGTP, and dUTP, etc.).

5x Buffer for rTth/ TTx (DNA/RNA)

Coming soon

5x Buffer for rTth/ TTx (DNA/RNA) <Glycerol Free>

5x Buffer for Tth/ TTx (DNA/RNA) is concentrated buffer components, consist of buffer and salts, used in *RNA-direct*™ Realtime PCR Master Mix (QRT-101). By mixing with Hot Start rTth DNA Polymerase, dNTPs, Mn^{2+} solution and ROX dye, QRT-101 can be reconstituted. This buffer system can be used with Hot start TTx DNA Polymerase.

5x Buffer for rTth/TTx (DNA/RNA) <Glycerol Free> is concentrated buffer components used in *RNA-direct*™ Realtime PCR Master Mix (QRT-101) except for glycerol which inhibit lyophilization. This buffer can be used with Hot start TTx DNA Polymerase <Glycerol Free> (Code No. HSTTX-229).

Store at -20 °C
Components:

Code No. QRT-1B1	40 ml
Code No. QRT-1B2	400 ml
5x Buffer for rTth/ TTx* (DNA/RNA)	
Code No. QRT-2BS	2 ml
Code No. QRT-2B1	20 ml
Code No. QRT-2B2	200 ml
10 x Buffer for rTth/ TTx (DNA/RNA) <Glycerol Free>*	
Code No. QRT-3BS	8 ml
Code No. QRT-3B1	80 ml
Code No. QRT-3B2	800 ml
2.5 x Buffer for rTth/ TTx (DNA/RNA) <Glycerol Free, Contain Excipient>*	

* Buffer doesn' t contain Mg^{2+}, Mn^{2+}, dNTPs and enzyme.

Features

- **The buffer can be used for the reconstitution of *RNA-direct*™ Realtime PCR Master Mix (Code No. QRT-101).**
- **Glycerol-free buffer can be used with TTx DNA Polymerase <Glycerol Free> (Code No. HSTTX-229) for lyophilization.**

Raw material related Tth/ TTx DNA Polymerase

50 mM $Mn(OAc)_2$

This product is used for 1 enzyme RT-PCR using Tth/ TTx DNA Polymerase.

Store at -20 °C
Components:

Code No. QRT-MN1

50 mM $Mn(OAc)_2$	5 ml

Features

- **The reagent can be used for the reconstitution of *RNA-direct*™ Realtime PCR Master Mix (Code No. QRT-101).**

25 mM $MgCl_2$

This product is used for PCR.

Store at -20 °C
Components:

Code No. TAP-2S1

25 mM $MgCl_2$	40 ml

Passive reference

50x ROX reference dye

50x ROX reference dye is used to normalize the fluorescent reporter signal between the wells in real-time quantitative PCR or RT-PCR.

Store at -20 °C
Components:

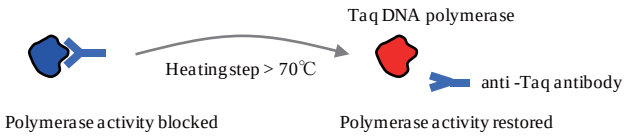
Code No. ROX-101

50x ROX reference dye	5 ml

instruments	ROX conc.
Applied Biosystems® 7000, 7300, 7700, 7900HT, StepOne™, StepOnePlus™	1x
Applied Biosystems® 7500, 7500Fast, ViiA™, QuantStudio®, Agilent Technologies Mx3000P, Mx3005P, Mx4000	0.1x

anti-Taq high

Anti-Taq DNA Polymerase Antibody 1, 2 <Glycerol Free>



anti-Taq high is a highly purified neutralizing monoclonal antibody to Taq and Tth 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 antibodies are quickly inactivated and PCR proceeds. The antibody-mediated hot start method is significantly more convenient to use than other hot start methods.

anti-Taq high contains two antibodies. Anti-Taq DNA Polymerase Antibody 1, 2<Glycerol Free> are glycerol-free antibodies of anti-Taq high.

Features

- **Enhances the specificity and sensitivity of PCR.**
- **Inhibits ≥95% of Taq DNA polymerase activity with anti-Taq high at 45ºC.**
- **No contamination of mouse genomic DNA, as determined by PCR.**
- **The polymerase can be reactivated quickly compared with a chemically modified polymerase.**

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.

Store at -20 °C

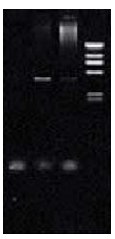
Components:

Code No. TCP-101	
anti-Taq high (1 mg/ml)*	100 µl
10x PCR buffer	1 ml
Code No. TCP-139	
anti-Taq high (1 mg/ml)*	30 ml
Code No. TCP-189CH1	
anti-Taq high (1 mg/ml)*	100 ml
Code No. TCP-309	
Anti-Taq DNA Polymerase Antibody 1 <Glycerol Free>**	1 mg
Code No. TCP-319	
Anti-Taq DNA Polymerase Antibody 1 <Glycerol Free>**	25 mg
Code No. TCP-409	
Anti-Taq DNA Polymerase Antibody 2 <Glycerol Free>**	1 mg
Code No. TCP-419	
Anti-Taq DNA Polymerase Antibody 2 <Glycerol Free>**	25 mg

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

** Storage buffer: 20 mM Tris-HCl (pH 7.5)

The concentration is different depending on Lot No.



M: λ/*Hin*d 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)

anti-Taq Neo

anti-Taq Neo <Glycerol Free>

Coming soon

anti-Taq Neo and anti-Taq Neo<Glycerol Free> are basic version of anti-Taq high. This products provide an antibody-mediated hot start PCR to enhance the specificity and sensitivity of PCR to neutralize Taq DNA Polymerase. anti-Taq Neo <Glycerol Free> is glycerol-free type of anti-Taq Neo.

- **Enhances the specificity and sensitivity of PCR.**
- **Inhibits ≥90% of Taq DNA polymerase activity at 45ºC.**
- **No contamination of mouse genomic DNA, as determined by PCR.**
- **The polymerase can be reactivated quickly compared with a chemically modified polymerase.**

Store at -20 °C

Components:

Code No. TCP-239	
anti-Taq Neo (1 mg/ml)	30 ml
Code No. TCP-259	
anti-Taq Neo (1 mg/ml)	100 ml
Code No. TCP-219GF	
anti-Taq Neo <Glycerol Free>	25 mg

Recombinant type RNase inhibitor

RNase Inhibitor, Recombinant

This product is a recombinant human placental ribonuclease inhibitor.
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. This inhibitor (51 kDa) exerts its inhibitory effect by noncovalently binding to RNases in a 1:1 ratio. This inhibitor can be applied to a reverse transcriptase reaction.

Store at -20 °C

Components:

Code No. SIN-201 2,500 U
Code No. SIN-229 100,000 U
Code No. SIN-259 1,000,000 U

RNase inhibitor (20-40 U/μl)

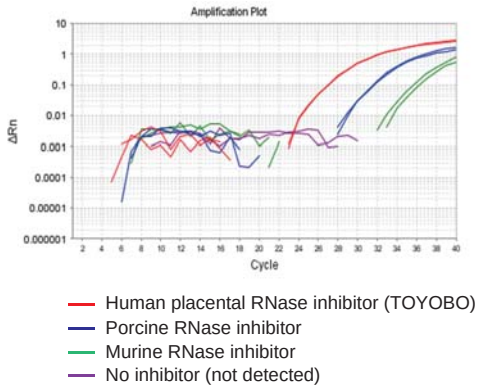
One unit is defined as the amount of RNase inhibitor required to inhibit the activity of 5 ng of ribonuclease A by 50%.

Applications

- cDNA synthesis, RT-PCR

Application data

1. Comparison of inhibitory effect between different types of RNase inhibitors



Cell lysates were prepared from 2.5 x 10^3 U937 cells in the presence of human placenta (TOYOBO), porcine and murine recombinant RNase inhibitors. Then, cDNA were synthesized using the lysates and β-actin genes were analyzed by real-time PCR.
The cDNA synthesized from the lysate with human placental RNase inhibitor showed better results than that with other RNase inhibitors.

Uracil-DNA Glycosylase

Uracil-DNA Glycosylase, Heat-labile
Uracil-DNA Glycosylase, Heat-labile <Glycerol Free>

Coming soon

Uracil-DNA Glycosylase, Heat-labile can be used with dUTP to eliminate carryover contamination from previous DNA synthesis reactions.
This enzyme is completely and irreversibly inactivated by moderate heat treatment (55°C) and does not degrade PCR products after PCR.
Uracil-DNA Glycosylase, Heat-labile <Glycerol Free> is glycerol-free type of Uracil-DNA Glycosylase, Heat-labile. The reagent can be used in a preparation of master mix reagents for lyophilization.

Store at -20 °C

Components:

Code No. UNG-101 200 U
Code No. UNG-109 10,000 U

Uracil-DNA Glycosylase, Heat-labile (1 U/μl)

Code No. UNG-201 200 U
Code No. UNG-209 10,000 U

Uracil-DNA Glycosylase, Heat-labile <Glycerol Free>

One unit of UNG is defined as the amount of enzyme required to release 1 nmol uracil from uracil-containing DNA per hour at 37°C.

Applications

- Carryover prevention

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 reduce 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.

Store at -20 °C
Components:

Code No. TRT-101 10,000 U

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

Code No. TRT-109 500,000 U

ReverTra Ace™ (100 U/μl)	5 ml

Features

- **RNase H minus M-MLV RTase with improved performance.**
- **Enables the synthesis of longer cDNAs (≥ 14 kb).**
- **Excellent reaction efficiency at high temperatures.**

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.

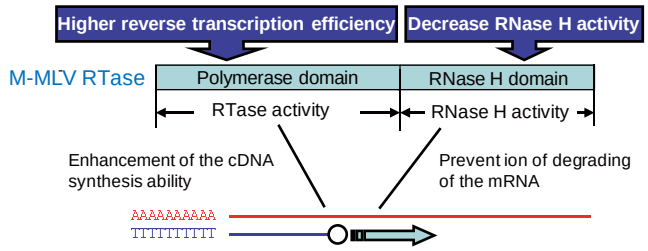


Fig. Genetically improved M-MLV Reverse transcriptase

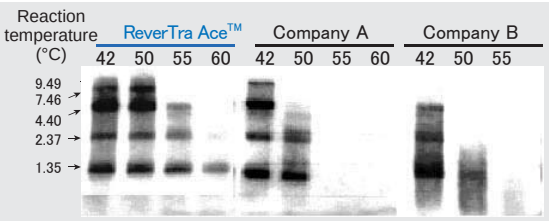


Fig. Comparison of elongation capability of RNase H minus RTases at various temperatures

Nucleotides

dNTP (dATP, dCTP, dGTP,dTTP, dUTP)
dNTPs Mixture
dNTPs Set

dNTPs Mixture is an equal mole solution mixture of ultrapure dATP, dCTP, dGTP, and dTTP, or dATP, dCTP, dGTP, and dUTP. dNTPs Set contains each dATP, dCTP, dGTP, and dTTP solution.

Applications

- PCR
- Reverse transcription

Store at -20 °C
Components:

Code No. NTP-101
dATP, dCTP, dGTP, dTTP (100 mM), 0.5 ml each

Code No. NTP-201
dNTPs Mixture (A, C, G, T each 2 mM), 1 ml

Code No. NTP-301
dNTPs Mixture (A, C, G, T each 10 mM), 0.2 ml

Code No. NTP-501
dNTPs Mixture (A, C, G, U each 2 mM), 1ml Coming soon

Code No. ATP-109
dATP (100 mM), 100 ml

Code No. CTP-109
dCTP (100 mM), 100 ml

Code No. GTP-109
dGTP (100 mM), 100 ml

Code No. TTP-109
dTTP (100 mM), 100 ml

Code No. UTP-101
dUTP (100mM), 500 μl Coming soon

Efficient heat-stable T7 RNA polymerase

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.

Store at -20 °C
Components:

Code No. TRL-201 7,500 U	
Thermo T7 RNA Polymerase (50 U/μl)	150 μl
10x Buffer*	2 x 1ml
Code No. TRL-252 50,000 U	
Thermo T7 RNA Polymerase (1,000 U/μl)	50 μl
10x Buffer*	3 x 1ml

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

Features

- **Increased enzyme activity compared with 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

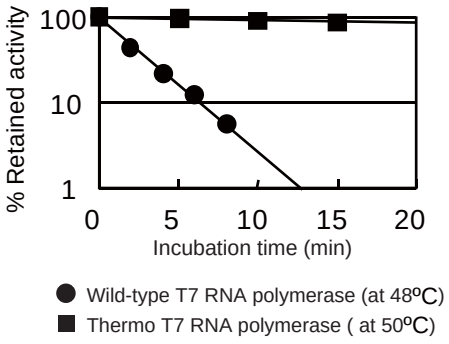
One unit is defined as the amount of enzyme that will incorporate 1 nmole of [^{3}H] rNTP into an acid insoluble material using T7 DNA as template in 60 min at 37ºC.

Application data

1. Comparison of heat stability

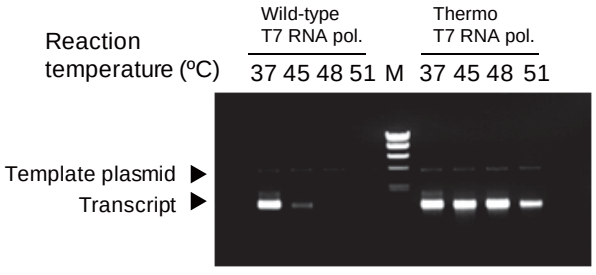
The residual activities of the wild-type T7 RNA Polymerase and Thermo T7 RNA polymerase were measured after incubation for various periods at 48ºC and 50ºC, respectively. As a result, Thermo T7 RNA polymerase retained its activity after incubation for 15 min whereas the activity of wild-type enzyme decreased 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. (at 48ºC.)
Thermo T7 RNA Polymerase: 84.5 min. (at 50ºC.)



2. *In vitro* transcription at high temperature

In vitro transcriptions were performed using wild type and Thermo T7 RNA Polymerases at various temperature conditions (37, 45, 48, 51ºC.).
The transcripts were detected by agarose gel electrophoresis. Distinct transcripts were detected from 37 to 51ºC with Thermo T7 RNA Polymerase.



Template:
Linearlized plasmid DNA carrying T7 promoter, 0.5 mg

Reaction time: 60 min.

High success-rate PCR enzyme

KOD One™ PCR Master Mix
KOD One™ PCR Master Mix -Blue-

KOD One™ PCR master Mix and KOD One™ PCR Master Mix -Blue- are 2 x PCR master mixes based on genetically modified KOD DNA polymerase (UKOD). KOD One™ series enables fast PCR, which has an extension time of 5 sec/ kb by applying UKOD and a new Elongation Accelerator. In addition, these master mixes provide greater efficiency and elongation capabilities than conventional PCR enzymes. In particular, they show greater amplification success from crude specimens. Furthermore, these master mixes can be applied to amplify from templates containing uracils (dU) or using primers containing inosines (dI) and uracils (dU).

KOD One™ series contains two types of anti-KOD DNA polymerase antibodies that inhibit the polymerase and 3'→ 5' exonuclease activities, thus allowing for Hot Start PCR. These master mixes generate blunt-end PCR products because of 3' → 5' exonuclease (proof-reading) activity of KOD DNA polymerase.

Store at -20 °C

Components:

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

KOD One™ PCR Master Mix	1 ml x 5

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

KOD One™ PCR Master Mix -Blue-	1 ml x 5

*The reagents can be stored at 4°C for a month. For longer storage, the reagents should be kept at -20°C

Features

- **Fast**
  KOD One™ series can amplify the targets using the following very short conditions:
  ≤1 kb: 1 sec
  1~ 10 kb: 5 sec/ kb
  10 kb~: 10 sec/ kb
  The cycling conditions can be set flexibly when various targets having different sizes are amplified.

- **Easy to Use**
  KOD One™ series contains all reaction components except for primers and templates and provide high reproducibility by reducing operations. In addition, KOD One™ PCR Master Mix -Blue- includes a loading dye (BPB) to allow direct loading onto agarose gels.

- **High Fidelity**
  KOD One™ series exhibits approximately 80-fold higher fidelity than Taq DNA polymerase. These mixes can be used for various purposes where this would be an advantage (e.g., in the preparation of long target amplicons for sequencing).

- **High Efficiency**
  KOD One™ series is effective for amplification from crude samples (e.g., biological samples, foodstuffs, soil extract, etc.). Various samples or lysates can be used directly as templates.

Applications

- Direct PCR
- Colony PCR
- Amplification of NGS libraries
- Site-directed gene mutation

Application data

1. Fast PCR

Various targets were amplified with KOD One™ PCR Master Mix and KOD One™ PCR Master Mix -Blue- using the fast cycling conditions. KOD One™ series successfully amplified all targets.

<Reaction Mix>

distilled water	21µl
KOD One™ PCR Master Mix	25µl
10pmol/µl Primer F	1.5µl
10pmol/µl Primer R	1.5µl
10ng/µl human genomic DNA	1µl
Total Volume	50µl

<PCR cycle>

Target length <1kb

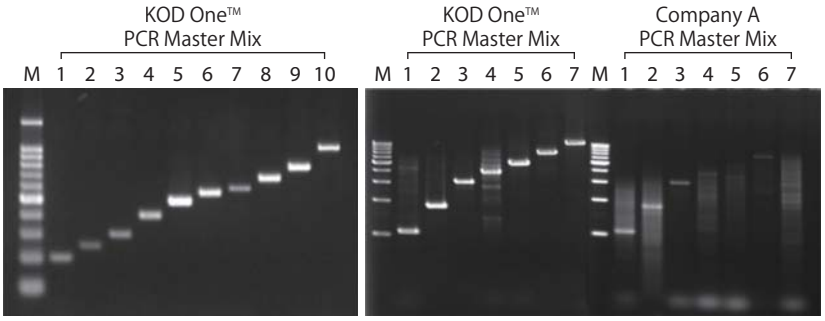
98℃ 10sec
60℃ 5sec
68℃ 1sec
X30 cycles

Target length 1-10kb

98℃ 10sec
60℃ 5sec
68℃ 5sec/kb
X30 cycles

Template: human genomic DNA

[Target length <1kb: Extension 1 sec 1-10 kb: Extension 5 sec/kb]



M : 100bp DNA Ladder
1 : DDB2(200bp)
2 : FANCG(250bp)
3 : HBg(300bp)
4 : CDH1(400bp)
5 : chrome9(500bp)
6 : ERCC4(550bp)
7 : HRAS(600bp)
8 : PRF1(700bp)
9 : BRCA1(800bp)
10 : CDK4(1,000bp)

M : 1kb DNA Ladder
1 : Chrome9(1kb)
2 : MSH6(2kb)
3 : FANCE(3kb)
4 : RAD51D(4kb)
5 : KRAS(5kb)
6 : BRCA1(7kb)
7 : DDB2(10kb)

2. Amplification efficiency and sensitivity

The 3.5 kb fragments were amplified from plasmid DNA and cDNA. Each PCR reaction was performed according to the recommended conditions. KOD One™ PCR Master Mix showed higher sensitivity using 5 sec/kb extension time.

<Reaction Mix>

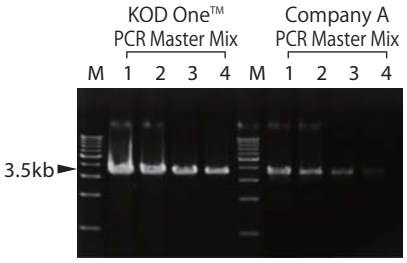
distilled water	21µl
KOD One™ PCR Master Mix	25µl
10pmol/µl Primer F	1.5µl
10pmol/µl Primer R	1.5µl
template	1µl
Total Volume	50µl

<PCR cycle>

98℃ 10sec
60℃ 5sec
68℃ 18sec (5sec/kb)
X30 cycles

[plasmid DNA]

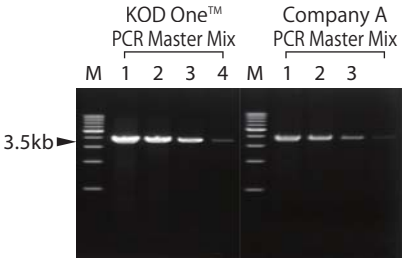
Template: plasmid DNA
Target: Polymerase gene



M : 1kb DNA Ladder
1 : 25 pg
2 : 6.3 pg
3 : 1.6 pg
4 : 0.4 pg

[cDNA]

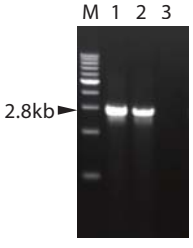
Template: cDNA from HeLa total RNA
Target: TFR



M : 1kb DNA Ladder
1 : 25 ng equivalent
2 : 5 ng equivalent
3 : 1 ng equivalent
4 : 0.2 ng equivalent

3. Amplification using degenerate primers containing inosine.

The 2.8 kb fragments were amplified using degenerate primers containing inosine. KOD One™ PCR Master Mix was able to amplify, whereas conventional high-fidelity PCR enzymes were not.



<Reaction Mix>

distilled water	17µl
KOD One™ PCR Master Mix	25µl
100pmol/µl Primer F	1.5µl*
100pmol/µl Primer R	1.5µl*
50ng/µl E.coli genomic DNA	5µl
Total Volume	50µl

<PCR cycle>

98℃ 10sec
60℃ 5sec
68℃ 15sec
X30 cycles

<Primer sequence>

Fwd: ATGGTICARATHCCICARAAY
Rev: RTGIGCYTGRTCCCARTTYTC

*In cases where degenerate primers are used, the primer concentrations should be increased depending on the degree of degeneracy.

M : 1kb DNA Ladder
1 : KOD One™ PCR Master Mix
2 : KOD One™ PCR Master Mix -Blue-
3 : KOD -Plus- Neo

Application data

4. Amplification from cDNA

The inhibitory effect of RNA in cDNA was compared using various PCR enzymes. KOD OneTM PCR Master Mix was not susceptible to RNA inhibition, and it was able to amplify targets under high concentrations of cDNA.

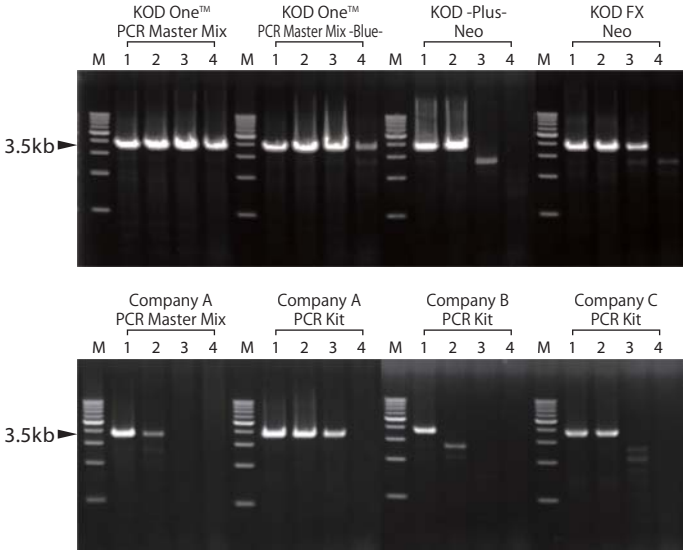
<Reaction Mix>

distilled water	17μl
KOD One™ PCR Master Mix	25μl
10pmol/μl Primer F	1.5μl
10pmol/μl Primer R	1.5μl
cDNA	5μl
Total Volume	50μl

<PCR cycle>

98℃ 10sec
60℃ 5sec
68℃ 18sec (5sec/kb)
X30 cycles

The PCR was performed according to the manufacturer's recommendations.

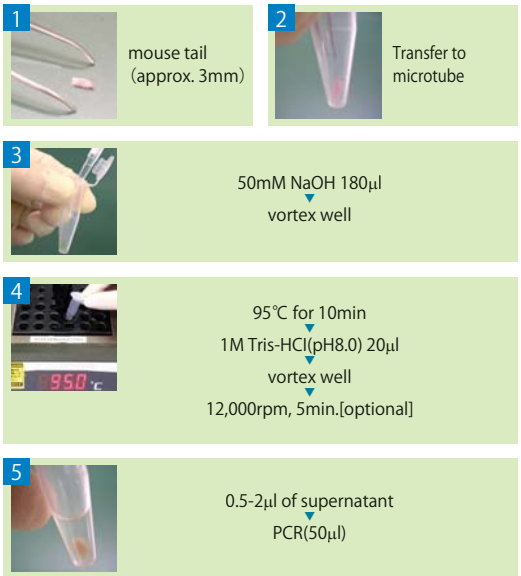


M : 1kb DNA Ladder
1 : 125 ng(RNA equivalent)
2 : 250 ng(RNA equivalent)
3 : 500 ng(RNA equivalent)
4 : 1,000 ng(RNA equivalent)

Template: cDNA from HeLa total RNA
Target: TFR

5. Amplification efficiency and sensitivity

Amplification from mouse lysate and whole blood were compared. KOD One™ PCR Master Mix amplified the targets efficiently.



<Reaction Mix>

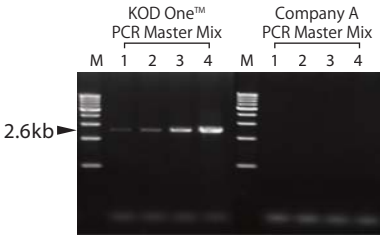
distilled water	Yμl
KOD One™ PCR Master Mix	25μl
10pmol/μl Primer F	1.5μl
10pmol/μl Primer R	1.5μl
template	Xμl
Total Volume	50μl

<PCR cycle>

98℃ 10sec
60℃ 5sec
68℃ 5sec /kb
X30 cycles

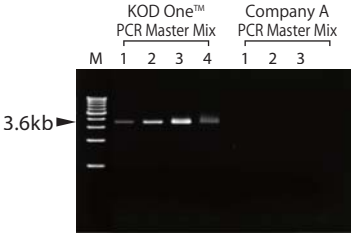
The PCR was performed according to the manufacturer's recommendations.

[Amplification from mouse lysate]



Template: supernatant
M:1kb DNA Ladder
1:1 μl 2:2 μl 3:4 μl 4:8 μl

[Direct amplification from whole blood]



Template: whole blood
M:1kb DNA Ladder
1:1 μl 2:2 μl 3:4 μl 4:8 μl

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.

Store at -20 °C

Components:

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

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

- **Effective amplification from crude samples.**
- **Direct amplification from various samples (whole blood, microorganisms).**
- **Effective amplification of difficult targets, such as high GC or AT content, and/or long targets.**
- **Effective amplification of long target.**
  The following amplifications is confirmed:
  40kb from phage lambda DNA, 24kb from human genomic DNA, and 13.5kb from cDNA.
- **Low error ratio, about 10 times less than that of Taq DNA polymerase.**
- **PCR products amplified using KOD FX is of blunt-end. TArget Clone™ -Plus- can be applied to the TA cloning.**

Applications

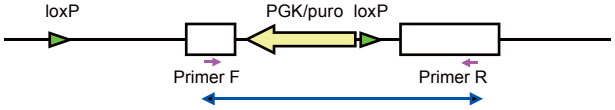
- Genotyping (Amplification from crude samples)
- Efficient amplification of difficult targets (high G/C, long) from genomic DNA or cDNA with fast mode
- High fidelity PCR

Application data

1. Comparison of PCR efficiency on mouse genotyping

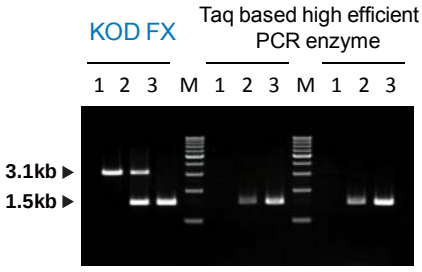
The target loci of the transgenic mice were amplified from the mouse tail lysates [see p13 Alkaline lysis methods] using KOD FX and the other Taq-based high efficient PCR enzymes. The target loci (3.1 kb) were successfully amplified by KOD FX.

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

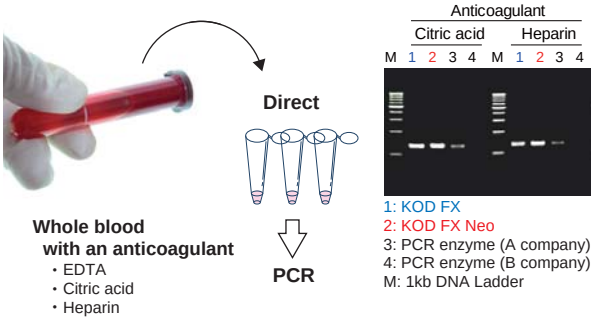
References

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

KOD FX 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

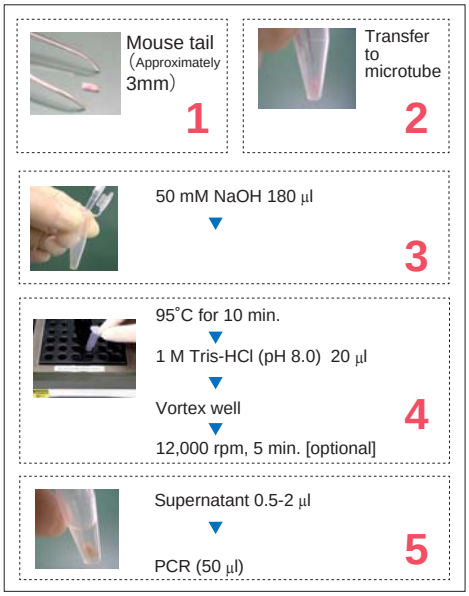


	X (μl)
DW	25
2x PCR Buffer	10
2 mM dNTPs	1.5
10 pmol/ml Primer F	1.5
10 pmol/ml Primer R	1
KOD FX or KOD FX Neo (1U/ml)	1~2*
Whole blood / Lysates etc.	
Total	50

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

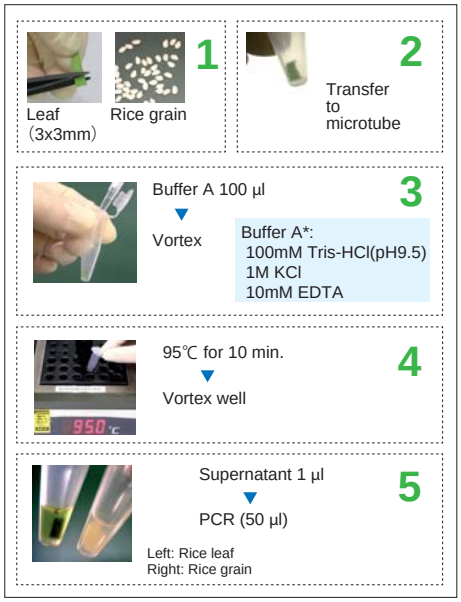
● Amplification from mouse tail lysates

Alkaline lysis method



● Amplification from plant samples

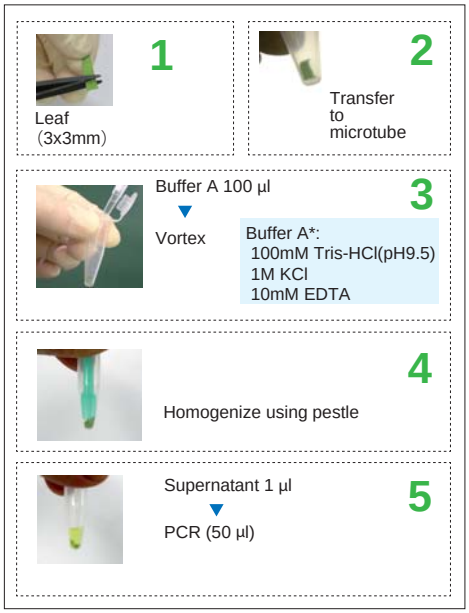
One step method



*BioTechniques, 19: 394 (1995)

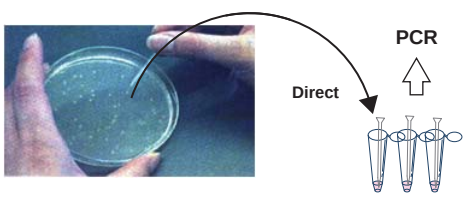
Homogenization method

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



*BioTechniques, 19: 394 (1995)

● Direct amplification from yeast & fungus colonies



GenNext™ NGS Library Prep Kit

The GenNext™ NGS Library Prep Kit designed for the rapid construction of libraries for Illumina® sequencing, from fragmented double-stranded DNA(dsDNA).
The GenNext™ NGS Library Prep Kit is designed for Library construction from a wide range of sample inputs (1ng-1μg).

The kit contains all of the enzymes and reaction buffers required for end repair and A-tailing, adapter ligation, and library amplification(optional).

Store at -20 °C

Components:

Code No. LPK-101T 8 reactions
LPK-101 24 reactions
LPK-101L 96 reactions

End Repair and A-tailing Buffer
End Repair and A-tailing Enzyme
Ligation Solution
Library Amplification Master Mix
Library Amplification Primer Mix
50x Dilution Buffer

*Adapter and SPRI (Solid Phase Reversible Immobilization) paramagnetic bead are not included in this kit.

Features

- **The kit performs end repair and A-tailing with an enzyme mix in a single tube.**
- **The kits are designed for library construction from a wide range of sample types and inputs (1 ng – 1 μg).**
- **Library Amplification Master Mix is designed for low-bias, high-fidelity PCR.**

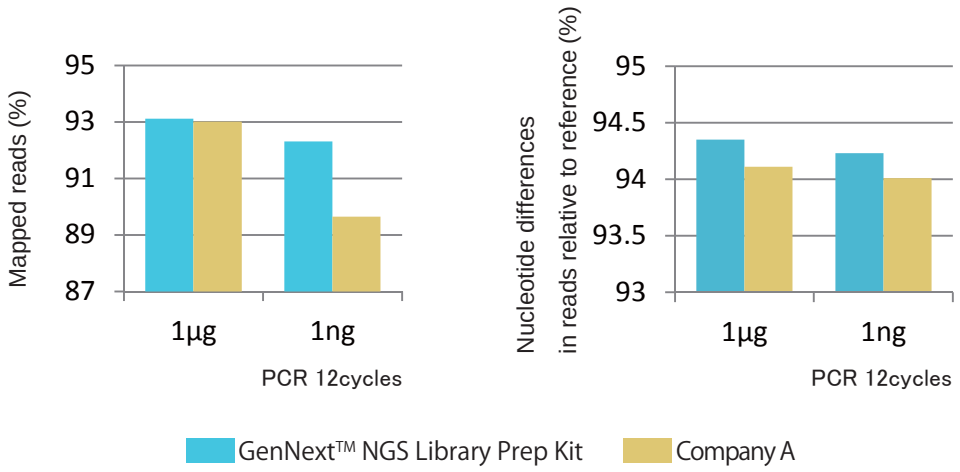
Applications

- **Library Preparation of Illumina® next-generation sequences**

Application data

1. Comparison of NGS accuracy with other manufacturers kit

We performed sequencing of *E. coli* genomic DNA using MiSeq and MiSeq Reagent Kit v2 (300 Cycles). CLC Genomics Workbench (Qiagen/CLC bio) was used for the sequence analysis, after the downsampling of each samples for 1 million leads.
GenNext™ Library Prep Kit provides superior results of mapped reads and matching rate with the reference.



NGS related product

GenNext™ NGS Library Quantification Kit

GenNext™ NGS Library Quantification Kit is for the SYBR® Green I qPCR-based library quantification of Illumina next-generation sequences. The kit allows the specific and accurate quantification of libraries bearing P5 and P7 adaptors which can be applied to flow cell amplification. It uses the highly efficient qPCR master mix KOD SYBR® qPCR Mix.

Store at -20 °C

Components:

Code No. NLQ-101 500 reactions

KOD SYBR® qPCR Mix	3 x 1.67 ml
50x ROX reference dye	250 µl
Standard DNA 1 (20 pM)	200 µl
Standard DNA 2 (2 pM)	200 µl
Standard DNA 3 (0.2 pM)	200 µl
Standard DNA 4 (0.02 pM)	200 µl
Standard DNA 5 (0.002 pM)	200 µl
Standard DNA 6 (0.0002 pM)	200 µl
5x Primer Mix	2 x 1 ml
50x Dilution Buffer	1.7 ml

Features

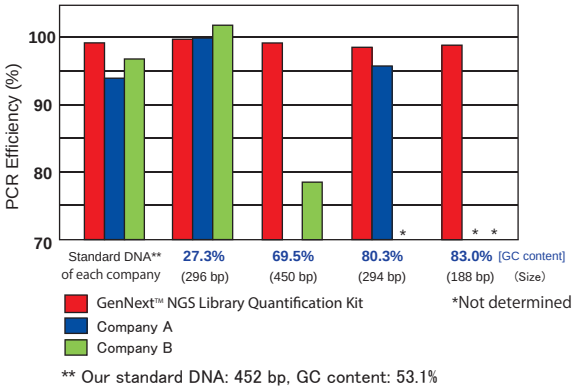
- **KOD SYBR® qPCR Mix can efficiently amplify GC- and AT-rich fragments of different lengths without bias.**
- **The kit has a broad dynamic range from 20 pM (Standard DNA 1) to 0.0002 pM (Standard DNA 6).**
- **The kit contains all reagents (KOD SYBR® qPCR Mix, 5X Primer Mix, Standard DNA, and 50X Dilution buffer) needed for the qPCR-based quantification of an NGS library.**

Applications

- qPCR-based library quantification of Illumina next-generation sequences

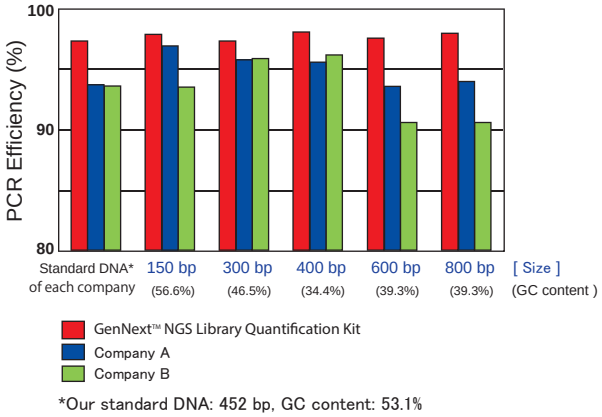
Application data

1. Comparison of PCR amplification efficiency of target genes with various GC contents.



Template DNAs of various GC contents bearing P5 and P7 adaptor sequences were analyzed. The GenNext™ NGS Library Quantification Kit achieved stable PCR efficiency with all targets, whereas other products showed poor specificity for targets with high GC contents.

2. Comparison of PCR amplification efficiency of target genes with various fragment sizes



Template DNAs of various fragment sizes (150–800 bp) bearing P5 and P7 adaptor sequences were analyzed. The GenNext™ NGS Library Quantification Kit achieved stable PCR efficiency with all targets, whereas other products showed poor efficiency with long targets.

东洋纺（上海）生物科技有限公司

上海市浦东新区张杨路 500 号华润时代广场 28 楼 AL 单元
邮编 200122
订货相关咨询
Tel: 021-5879-4900 Fax: 021-5879-4901
E-mail：market@bio-toyobo.cn
技术相关咨询
Tel: 021-5879-4142 Fax: 021-5879-5259

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