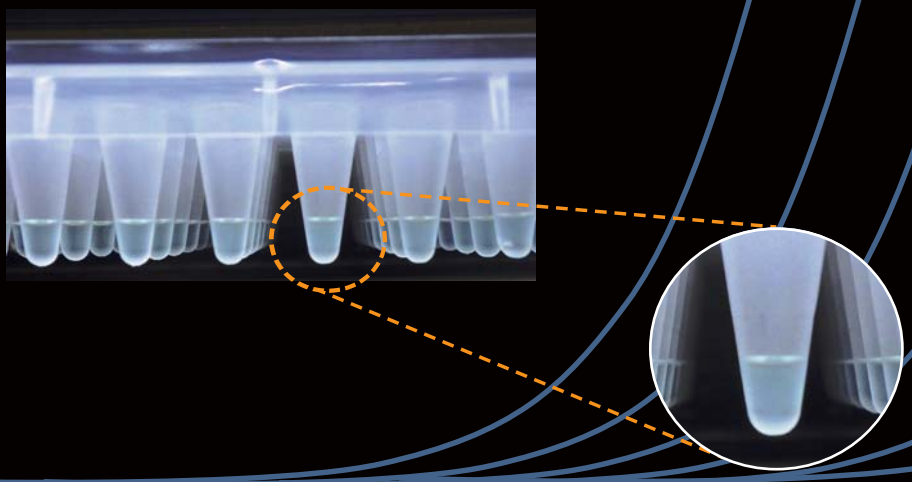
TOYOBO

THUNDERBIRD™ Next SYBR® qPCR Mix

- High amplification efficiency
- High sensitivity of Low copy areas
- High specificity
- Corresponding to high-speed cycle (10 sec. extension)
- Improving the stability of mixed reaction solution
- Prevention of false positives due to carry-over
  (combined with separately available UNG)
- Broad instrument compatibility
- Colored master mix

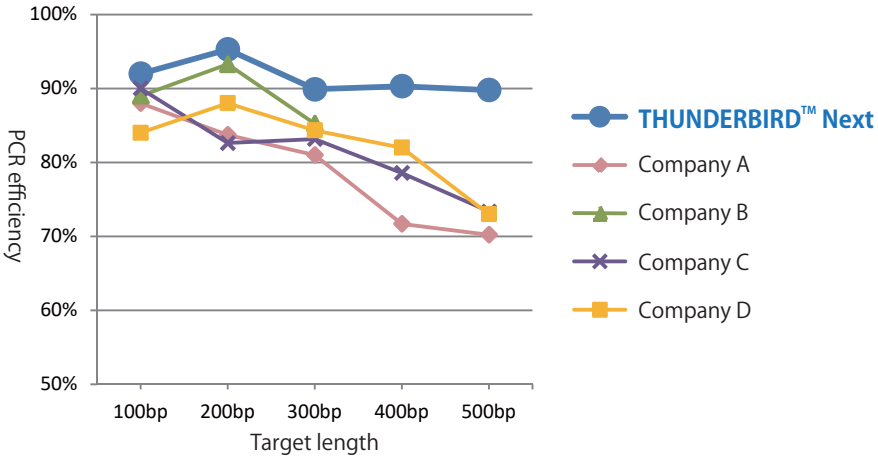


Feature 1 ：High amplification efficiency

THUNDERBIRD™ Next SYBR® qPCR Mix allows efficient amplification up to 500bp with minimal variability in PCR efficiency per target.

【Amplification of 100bp to 500bp target】

Using artificial gene as template and forward primer as common, reverse primer was designed so that the amplification product lengths were 100bp, 200bp, 300bp, 400bp and 500bp. We used these primers to amplify 10^7 to 10 copies using THUNDERBIRD™ Next SYBR® qPCR Mix and other companies to compare the PCR efficiency for each targets. As a result, the amplification efficiency may decrease or be undetectable as the target length increases in other companies, but stable PCR efficiency was achieved with THUNDERBIRD™ Next SYBR® qPCR Mix.

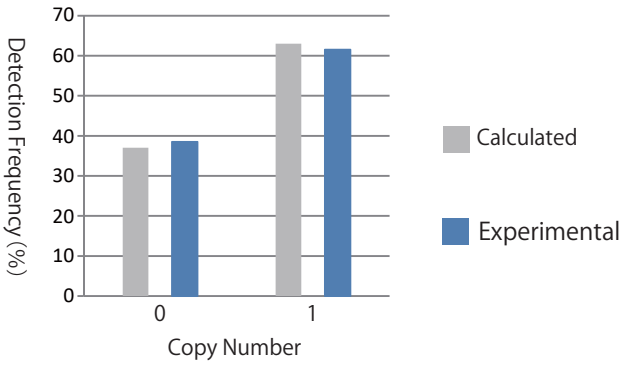


Feature 2 ：High sensitivity of Low copy areas

High efficiency and specific amplification to low copies is possible, allowing analysis with a wide measurement range.

【Single copy detection】

If one copy target can be amplified, the number of detected copies would be equivalent to the expected number of detected copies from the Poisson distribution. When one copy is added, the theoretical value from the Poisson distribution is 37% of the probability of having 0 copies and 63% of the probability of having one or more copies.
THUNDERBIRD™ Next SYBR® qPCR Mix was used to detect 96 samples using Salmonella genome diluted to one copy as a template.
The results showed that 38.5% of the samples were undetected and 61.5% were detected, which is equivalent to the expected number of samples from the Poisson distribution, suggesting that one copy equivalent can be detected.



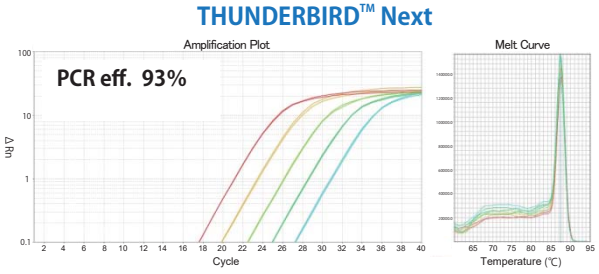
Feature 3 ：High specificity

Reducing non-specific reactions improves the reliability of detecting low-concentration targets.

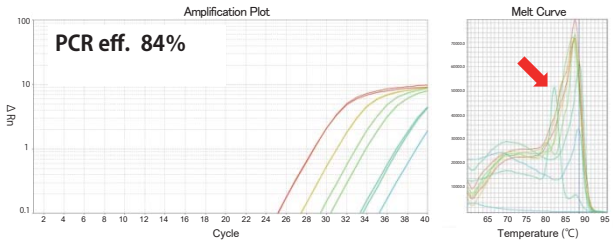
【Amplification of G3PDH gene (65bp)】

cDNA from total RNA of Hela cells synthesized by reverse-transcription reagents (Code No. FSQ-101) was used to amplify a 65-bp G3PDH gene at 5-fold dilutions of cDNA. As a result, nonspecific amplification occurred in the low copy range in other companies, but nonspecific amplification was not observed by using THUNDERBIRD™ Next SYBR® qPCR Mix, allowing accurate quantification to the low copy range.

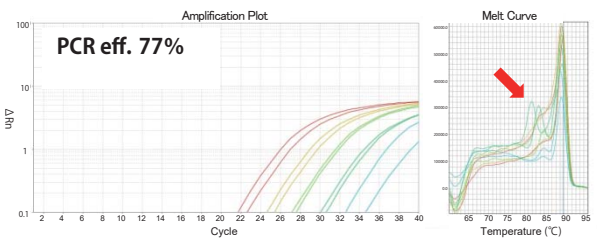
THUNDERBIRD™ Next



Company B



Company C

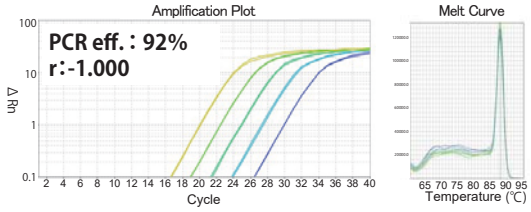
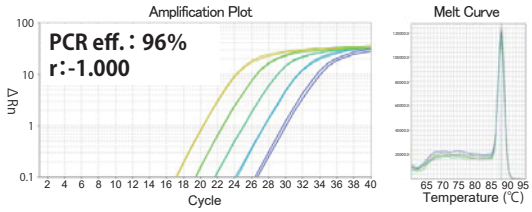
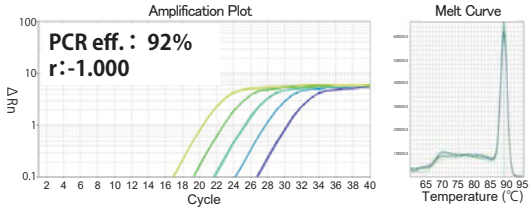
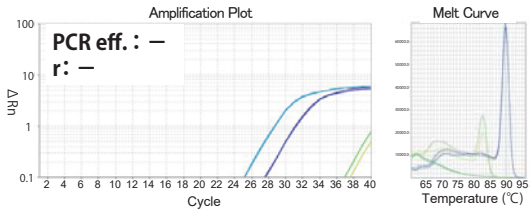


Feature 4 ：Corresponding to high-speed cycle

THUNDERBIRD™ Next SYBR® qPCR Mix can also be applicable by high-speed PCR with an extension time of 10 seconds.

【Comparison of Normal cycle and High-speed cycle】

Using cDNA from Hela cells total RNA synthesized by reverse-transcription reagents (Code No. FSQ-101), amplification of β-actin gene (316bp) was amplified by "normal cycle with 30sec. extension " and "high-speed cycle with 10sec. extension". As a result, other company products that recommend normal cycling could not be amplified efficiently by high-speed cycling, but THUNDERBIRD™ Next SYBR® qPCR Mix could be amplified efficiently by high-speed cycling.

	【Normal cycle】	【High-speed cycle】
THUNDERBIRD™ Next		
Company D		
	95℃, 60sec. ↓ 95℃, 15sec. / 60℃ , 30sec. (40 cycles) ↓ Melting analysis	95℃, 30sec. ↓ 95℃, 3sec. / 60℃ , 10sec. (40 cycles) ↓ Melting analysis

Feature 5 ：High stability of mixed solution

Stable results are obtained due to high stability when primer and templates are mixed.

【Stability of mixed solution】

Primers and template (cDNA from Hela cellular total RNA) were mixed into the PCR-reaction solution, and amplifications of the targets were performed immediately or after standing for 48 hours at a light-shielding room temperature. As a result, Ct values decreased after 48 hours in our conventional kit and other products. But in THUNDERBIRD™ Next SYBR® qPCR Mix, Ct values remained stable even after 48 hours.

THUNDERBIRD™ Next

Target gene	Ct, 0hr	Ct, 48hr	ΔCt
G3PDH	26.61	26.63	0.02
ACTB	23.72	23.85	0.13
GNB2L1	24.63	24.66	0.03
PBGD	31.36	31.00	-0.36
ABL1	28.69	28.38	-0.31
B2M	23.58	23.56	-0.02
RPL32	24.11	23.97	-0.14
TUBB	31.51	31.05	-0.46

Conventional kit

Target gene	Ct, 0hr	Ct, 48hr	ΔCt
G3PDH	30.38	31.17	0.79
ACTB	23.43	23.44	0.01
GNB2L1	24.01	24.68	0.67
PBGD	31.27	31.01	-0.26
ABL1	28.45	30.18	1.73
B2M	22.83	23.05	0.22
RPL32	23.56	24.11	0.55
TUBB	33.64	34.88	1.24

Company A

Target gene	Ct, 0hr	Ct, 48hr	ΔCt
G3PDH	28.83	29.16	0.33
ACTB	23.45	25.12	1.67
GNB2L1	23.83	24.71	0.88
PBGD	29.95	30.98	1.03
ABL1	28.19	28.75	0.56
B2M	22.65	22.72	0.07
RPL32	23.67	23.42	-0.25
TUBB	31.54	28.12	-3.42

Company B

Target gene	Ct, 0hr	Ct, 48hr	ΔCt
G3PDH	28.17	30.32	2.15
ACTB	25.02	26.25	1.23
GNB2L1	25.82	26.64	0.82
PBGD	32.35	33.38	1.03
ABL1	30.46	31.23	0.77
B2M	22.65	22.72	0.07
RPL32	25.41	26.11	0.7
TUBB	30.92	31.75	0.83

Differences with ΔCt greater than or equal to 0.5 are shown in yellow.

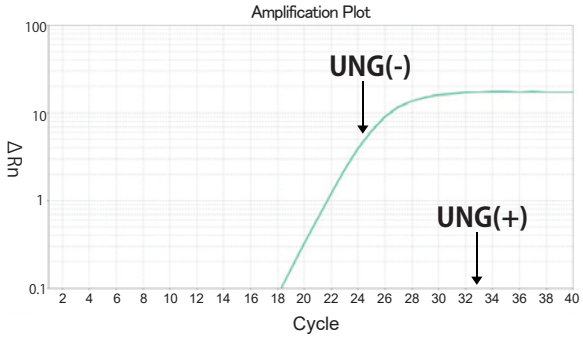
Feature 6 ：Prevention of false positives

This product includes dUTP. Therefore, false positives due to carry-over contamination can be prevented by using Uracil-DNA Glycosylase (UNG).

【Prevention of false positive with UNG treatment 】

To confirm UNG treatment for preventing carry-over contamination. PCR products containing dUTP (10^4 copies) were used as template, THUNDERBIRD™ Next SYBR® qPCR Mix and Uracil-DNA Glycosylase(UNG), Heat-labile (Code No. UNG-101) were added, and amplification of the same target was performed by real-time PCR. As a result, we were able to confirm that the first PCR products were degraded by UNG treatment completely.

Product	Code No.
Uracil-DNA Glycosylase(UNG), Heat-labile (200U×1)	UNG-101



Feature 7 ：Broad instrument compatibility

As this product is already mixed with universal passive reference dye, any instruments can be used with same reagent. Therefore, it is not necessary to mix ROX or use some reagents according to the real-time PCR instrument.

Example of available devices

Applied Biosystems	7300 / 7500 / 7500 Fast / StepOne / StepOnePlus ViiA 7 / QuantStudio
Roche Diagnostics	LightCycler 1. x / 2.0 / Nano / 96 / 480
Bio-Rad/MJ	MiniOpticon / CFX96 Touch
Agilent Technologies	Mx3000 / Mx3005P / Mx4000 / AriaMx
TaKaRa	Dice / DiceII / Dice Lite / Dice III
QIAGEN	Rotor-Gene Q
BioFlux	Line Gene

Feature 8 ：Colored master mix

The composition of this product contains blue dye. The dispensed wells of this product are blue, so dispensing errors can be reduced.

The blue dye does not affect the PCR reaction, detection, or stability.

Product	Reaction	Store	Code No.
THUNDERBIRD™ Next SYBR® qPCR Mix	500 reactions (1.67mL × 3tubes)	-20℃	QPX-201

＊This product is 2× Master Mix. It can be used for 500 reactions in 20μL reaction.

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