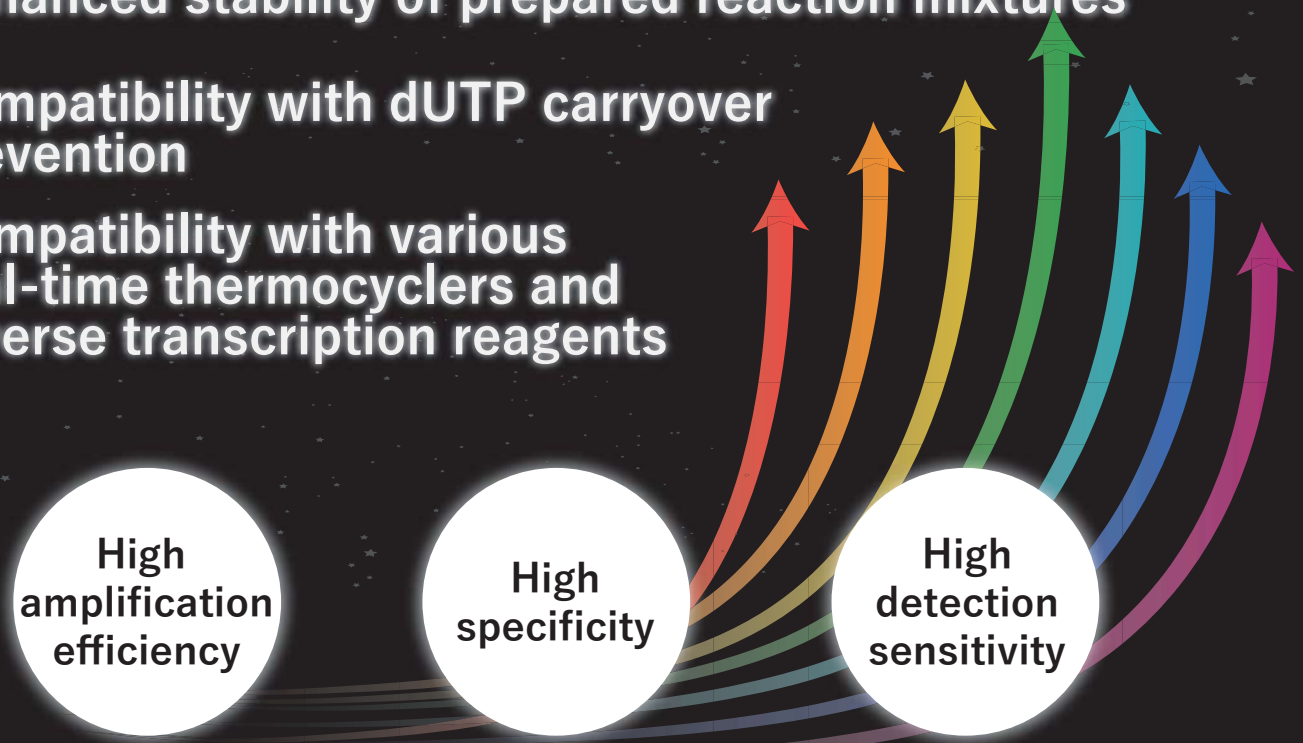
TOYOBO

High efficiency real-time PCR Master Mix

THUNDERBIRD™ Next Probe qPCR Mix

This product exhibits high amplification efficiency, specificity, and detection sensitivity, and enables fast and high-efficiency amplification over a broad dynamic range!

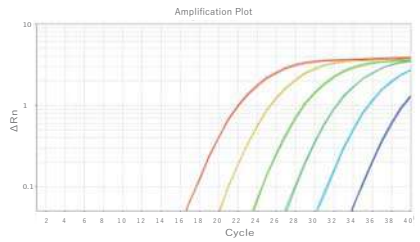
- Broad dynamic range
- Fast cycles with 10 s extension times achievable
- Improved quantitative performance in the presence of PCR inhibitors
- Enhanced multiplex detection performance
- Enhanced stability of prepared reaction mixtures
- Compatibility with dUTP carryover prevention
- Compatibility with various real-time thermocyclers and reverse transcription reagents

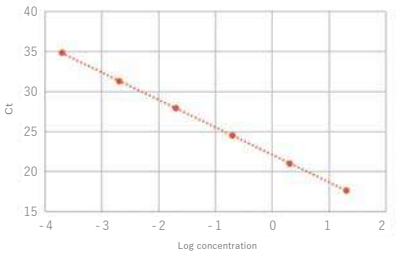


Feature 1 Broad dynamic range

High efficiency and specific amplification enable analysis over a broad dynamic range.

GAPDH was detected using a fluorescent dye (FAM) labeled TaqMan® probe.
cDNA from HeLa cell total RNA (total RNA 0.2 pg ~ 20 ng equivalent) was used.
Analyses confirmed high quantitative performance over a broad dynamic range from 0.2 pg to 20 ng.



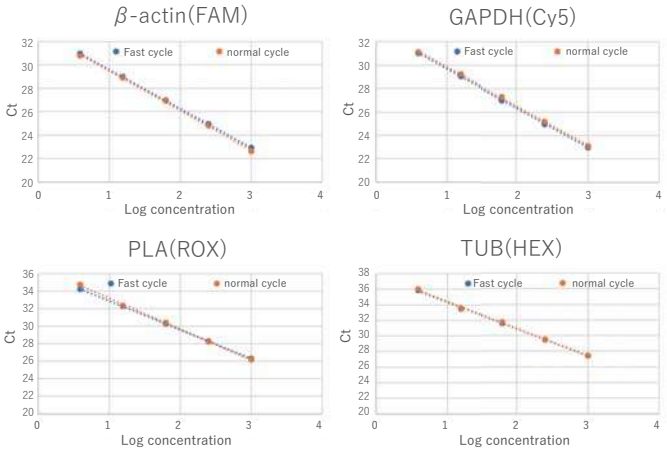


GAPDH(FAM)	
PCR efficiency	R^2
95.5%	1.000

Feature 2 Fast cycles of 10 s extension times achievable

Targets can be detected even in fast PCR with an extension time of 10 s.

β-actin, GAPDH, PLA, and TUB genes were detected using a multiplex detection system with TaqMan® probes labeled with four different fluorescent dyes in a "Normal cycle" with an extension time of 30 s and in a "Fast cycle" with an extension time of 10 s.
4-fold dilutions [5 steps] of cDNA derived from HeLa cell total RNA were used as samples and analyzed using Bio-Rad CFX96.
High PCR efficiency was demonstrated in fast cycles, even in multiplex detection system where amplification is difficult.



	β-actin (FAM)		GAPDH (Cy5)		PLA (ROX)		TUB (HEX)	
Cycling	PCR efficiency	R^2	PCR efficiency	R^2	PCR efficiency	R^2	PCR efficiency	R^2
Fast	99.2%	0.997	98.7%	0.999	100.8%	0.998	94.4%	0.996
Normal	96.7%	0.998	99.3%	0.999	91.7%	0.998	92.3%	0.996

Feature 3 Enhanced stability of prepared reaction mixtures

Our unique Buffer composition improves the stability of primer-template mixtures.

After mixing primers, probes, and templates in PCR mixtures, target amplification was performed immediately or after incubation for 48 hours at room temperature, protected from light.
Ct value delayed after 48 hours with other companies' products; however, THUNDERBIRD™ Next Probe qPCR Mix showed stable performance even after 48 hours. If there is a difference in ΔCt of 0.5 or more, or if ΔCt cannot be detected, the column is highlighted in yellow.

THUNDERBIRD™ Next Probe qPCR Mix

Target	Ct (0hr)	Ct (48hr)	ΔCt
Salmonella	31.11	31.48	0.37
Shigella	31.98	31.82	-0.16
VTEC	31.90	31.80	-0.10

Company A Real-time PCR Reagent (1)

Target	Ct (0hr)	Ct (48hr)	ΔCt
Salmonella	33.24	34.36	1.12
Shigella	32.16	33.30	1.14
VTEC	32.02	32.34	0.32

Company A Real-time PCR Reagent (2)

Target	Ct (0hr)	Ct (48hr)	ΔCt
Salmonella	32.34	N/A	—
Shigella	32.93	N/A	—
VTEC	32.04	31.9	-0.14

Company B Real-time PCR Reagent

Target	Ct (0hr)	Ct (48hr)	ΔCt
Salmonella	N/A	N/A	—
Shigella	33.22	N/A	—
VTEC	32.41	N/A	—

N/A : Not Amplified

Feature 4 Improved Quantitative performance in the presence of PCR inhibitors

Efficient amplification can be performed even in the presence of PCR inhibitors.

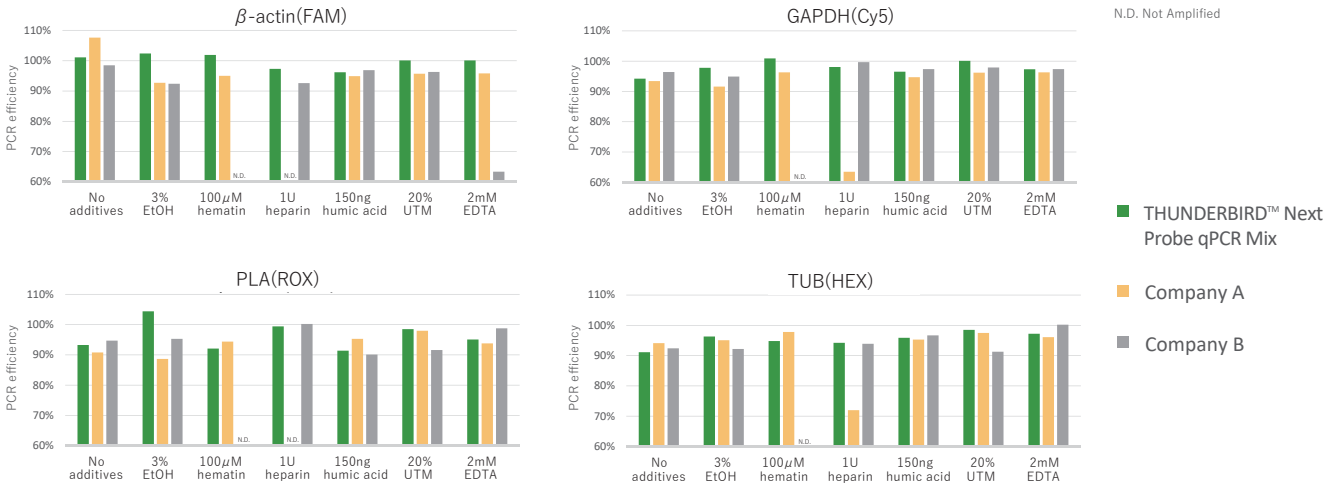
Feature 5 Enhanced multiplex detection performance

Efficient multiplex detection amplification can be performed.

<Example 1>

β-actin, GAPDH, PLA, and TUB genes were detected using a multiplex detection system with TaqMan® probes labeled with four different fluorescent dyes in the presence of the inhibitors described in the figure below.

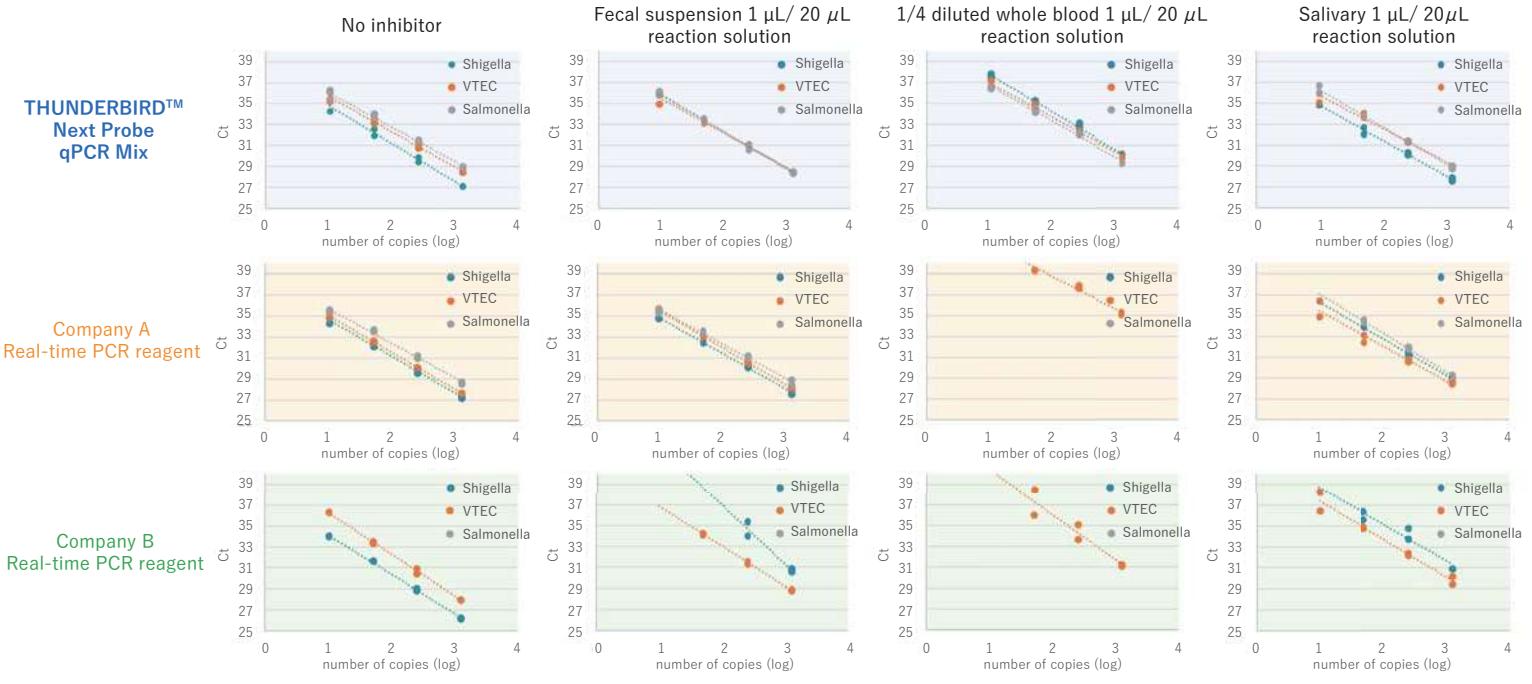
4-fold dilutions [5 steps] of cDNA from HeLa cell total RNA were used as samples and analyzed using Bio-Rad's CFX96. Addition of PCR inhibitors caused amplification failure with other companies' reagents. By contrast, using THUNDERBIRD™ Next Probe qPCR Mix, no significant difference in efficiency was observed even when inhibitors were added.



<Example 2 >

gDNA derived from Shigella, VTEC, and Salmonella were detected using a multiplex detection system with TaqMan® probes labeled with four different fluorescent dyes. These targets were detected in the presence of PCR inhibitors (feces, whole blood, and saliva). These detections were performed using Bio-Rad's CFX96.

While other companies' reagents cannot efficiently amplify in a multiplex detection system in the presence of PCR inhibitors, THUNDERBIRD™ Next Probe qPCR Mix can detect as low as 10 copies of each bacterial gDNA even in the presence of PCR inhibitors.

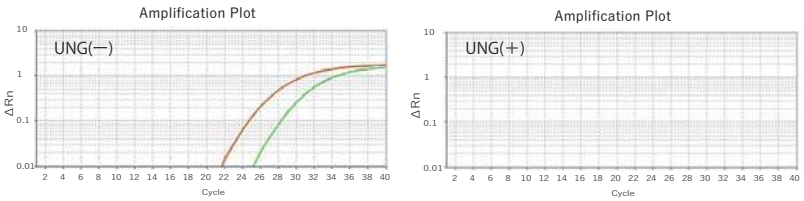


Feature 6 Compatibility with dUTP carryover prevention

The use of dUTP prevents false positives due to carry over contamination.

Using enterovirus RNA as templates, a 196 bp target was amplified with RT-PCR reagents containing dUTP. The PCR products (10^4 copies and 10^3 copies) were used as templates, and THUNDERBIRD™ Next Probe qPCR Mix, Uracil-DNA Glycosylase (UNG), Heat-labile [Code No. UNG-101] were added. The same target as in the first PCR was amplified using real-time PCR. The first PCR product was degraded using UNG treatment, and no amplification was observed in the second PCR.

※Uracil-DNA Glycosylase (UNG) is not supplied with this kit.
Uracil-DNA Glycosylase (UNG), Heat-labile [Code No.UNG-101] , sold separately, is required.



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

Feature 7 Compatibility with various real-time cyclers and RT reagents

This kit can be used with various real-time PCR instruments and RT reagents.

This kit can be used with typical real-time PCR instruments, and can be combined with various RT reagents. This kit combined with Toyobo's RT reagents (ReverTra Ace™ series and *SuperPrep*™ series) has been shown to enable highly accurate and sensitive expression analysis.

Feature comparison table

<Real-time PCR>

Template	Product Name	Code No.	Fast cycle	dUTP contain*	Passive reference contain	Specificity	Efficiency	Multiplex detection	Easy to use	Amplification from crude samples
DNA	THUNDERBIRD™ Next Probe qPCR Mix	QPX-101	✓	✓	✓	+++	+++	+++	+++	+++
	THUNDERBIRD™ Next SYBR™ qPCR Mix	QPX-201	✓	✓	✓	+++	++	+	+++	++
	Hot Start TTx (DNA) Kit	HSTTX-101	✓	✓		++	+++	++	+	+++
RNA	THUNDERBIRD™ Probe One-step qRT-PCR Master Mix	QRZ-101	✓	✓	✓	+++	+++	+++	++	+++
	RNA-direct™ SYBR™ Green Realtime PCR Master Mix	QRT-101			✓	+	++	+	++	+
	Hot Start TTx (RNA) Kit	HSTTX-111	✓			+	++	++	+	+++

* Adding Uracil-N-Glycosylase(UNG) can prevent false positives due to carry-over contamination.

<cDNA Synthesis>

Product Name	Code No.	Application	Efficiency	Master Mix	Genomic DNA removal	Trace RNA / Single cell
ReverTra Ace™ qPCR RT Kit	FSQ-101	qPCR from purified RNA	++			
ReverTra Ace™ qPCR RT Master Mix	FSQ-201	qPCR from purified RNA	++	✓		
ReverTra Ace™ qPCR RT Master Mix with gDNA Remover	FSQ-301	qPCR from purified RNA	++	✓	✓	
SuperPrep™ II Cell Lysis & RT Kit for qPCR	SCQ-401	qPCR from cultured cells	++	✓	✓	
QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit	RMQ-101	qPCR from purified trace RNA or single cell	+++		✓	✓

+++	BEST
++	Excellent
+	Good
✓	Applicable

TOYOBO TOYOBO CO.,LTD.

Biotechnology Overseas Sales and Marketing Department
Osaka Umeda Twin Towers South, 1-13-1 Umeda, Kita-ku,
Osaka, 530-0001 Japan
(Mail) bio_overseas@toyobo.jp
(URL) https://www.toyobo-global.com/seihin/xr/lifescience/

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