4×Lyo-Ready One-step qRT-PCR Master Mix with Excipient

THUNDERBIRD™ Next Probe One-step qRT-PCR 4×Mix (Lyo-Ready Mix)



Feature 1: Rapid and Sensitive
This corresponds to a fast cycle.
It enables rapid and sensitive detection of trace amounts of RNA.

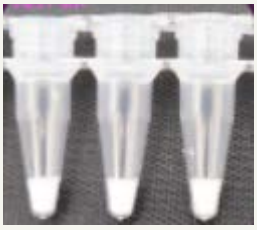
Feature 2: Easy to use
The primers, probes, and samples are simply added to initiate the reaction.
This reduces the risk of contamination and pipetting errors.

Feature 3: Improving contaminant resistance
Efficient amplification can be achieved even in the presence of contaminants that inhibit PCR.

Feature 4: High multiplex performance
This corresponds to the multiplex reactions that can simultaneously detect multiple genes.

Feature 5: Prevention of false positives by carryover
(combined with UNG sold separately)
The master mix contains dUTP.
This can prevent false positives due to carryover contamination.

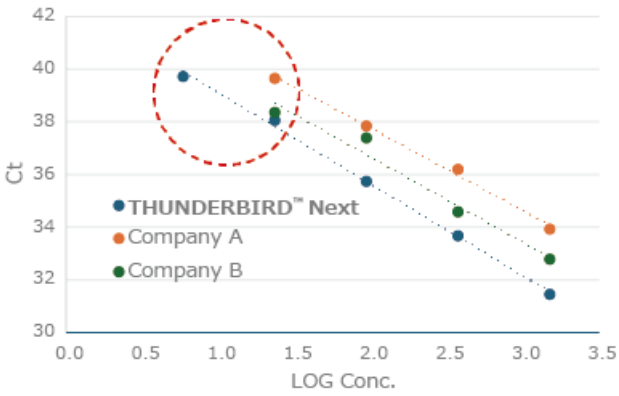
Feature 6: Enables lyophilization
Excipients for lyophilization are included, allowing for the preparation of lyophilized reagents.


Example of a lyophilized reaction mix

RNA detection and quantification made easier and more efficient!

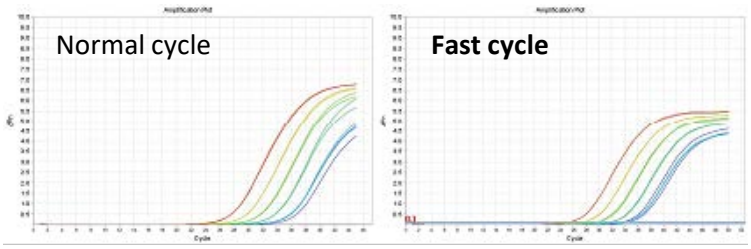
Example 1: Detection sensitivity comparison

Using TaqMan™ probe, we compared the detection sensitivity and quantitation of mumps virus RNA with other companies' products.
Only this kit was able to detect RNA of less than 10 copies and quantify it over a wide dynamic range. This kit is effective in detecting RNA viruses and mRNAs with low expression levels.



Example 2: Detection comparison during fast cycles

Influenza virus RNA was detected using the “Normal” cycle with an elongation time of 45 seconds and the “Fast” cycle with an elongation time of 10 seconds. Analysis was performed using Applied Biosystems™ StepOnePlus™.
As a result, efficient amplification was possible even with a fast cycle of 10 s elongation time.



	Normal cycle	**Fast cycle**
PCR efficiency	93.3%	90.2%
r	-0.998	-0.998

Normal cycle

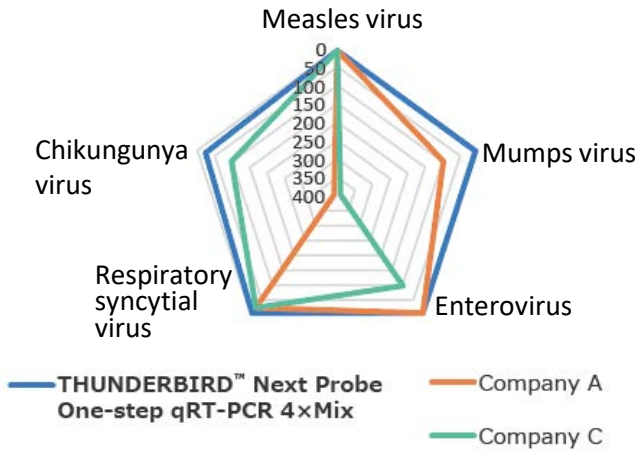
50 ℃ 10 min
95 ℃ 1 min
↓
95 ℃ 15 sec
55 ℃ 45 sec ×45 cycles

Fast cycle

50 ℃ 10 min
95 ℃ 30 sec
↓
95 ℃ 5 sec
55 ℃ 10 sec ×45 cycles

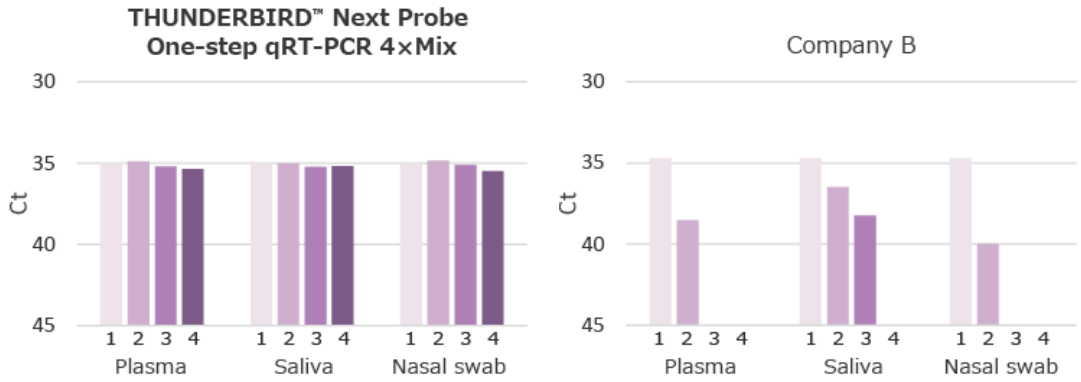
Example 3: Comparison of the detection sensitivities for different viruses.

The detection sensitivity of five types of viral RNA were cmpared using TaqMan™ probes. Applied Biosystems™ StepOnePlus™ was used for the analysis.
This product was able to detect all targets with a sensitivity of 10 copies or less.
It is possible to detect various RNAs with high sensitivity, regardless of the primer/probe sequence.



Example 4: Tolerant of contaminant

Biological samples (nasal swabs, saliva, and plasma) were added to the reaction to detect 50 copies of influenza virus RNA and evaluate resistance to PCR inhibitors. Bio-Rad CFX96 Touch Deep Well was used for the analysis.
While other company reagents caused poor amplification when PCR inhibitors were added, this reagent showed minimal effect on Ct even when biological samples were added.

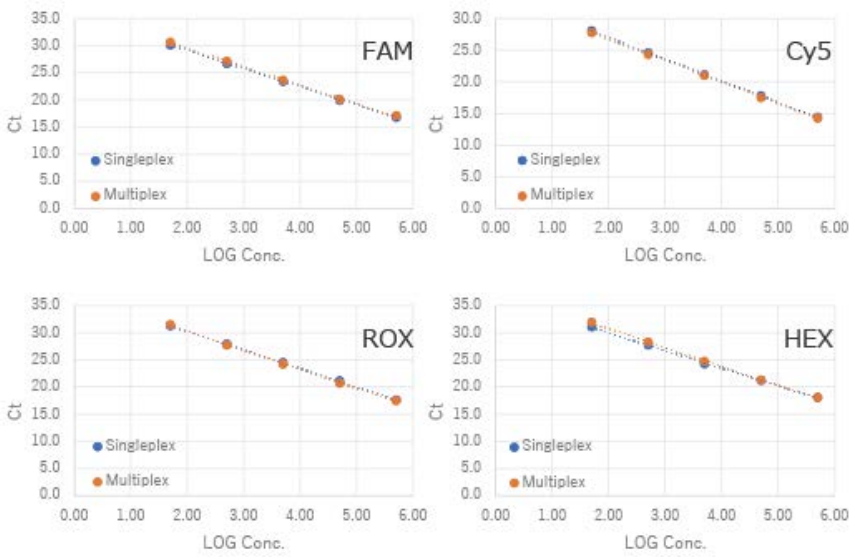


Amount of added contaminants (final concentration)

	Plasma	Saliva	Nasal swab
1	No addition	No addition	No addition
2	1%	1%	15%
3	2%	2%	20%
4	4%	4%	25%

Example 5: Comparison between singleplex and multiplex methods

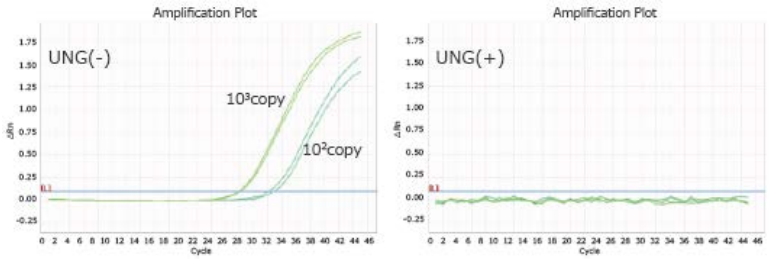
Single-and multiplex detections were compared using TaqMan™ probes labeled with four different fluorescent dyes. HeLa S3 RNA (10x dilutions of 500 ng to 0.05 ng [5 steps]) was used as a template.
The results revealed that single and multiplex detection showed comparable PCR efficiencies and that multiplex detection did not affect the reaction.



fluorescent dyes	FAM		Cy5		ROX		HEX	
Target	β-actin		GAPDH		PLA		TUB	
	Singleplex	Multiplex	Singleplex	Multiplex	Singleplex	Multiplex	Singleplex	Multiplex
PCR efficiency	98.8%	96.5%	96.5%	97.5%	97.0%	92.9%	103.1%	93.9%
r	-1.000	-0.999	-1.000	-1.000	-1.000	-0.999	-1.000	-0.999

Example 6: Carryover prevention by adding UNG

Assuming that the splashed amplified product was contaminated in the subsequent PCR (carryover contamination), we used the amplified sample (196bp) equivalent to 10^3, 10^2 copies as a template and detected the same target under the conditions of UNG addition (+)/(-).



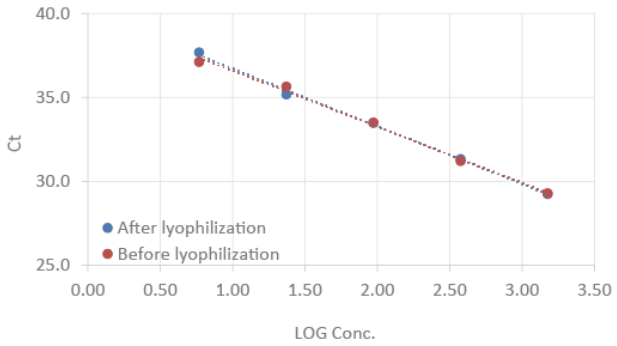
Analysis was performed using Applied Biosystems™ StepOnePlus™.
As a result, the template was degraded and no amplification was observed when UNG was added.

Example 7 : Performance comparison before and after lyophilization

qPCR Mix was prepared using THUNDERBIRD™ Next Probe One-step qRT-PCR 4 × Mix, and 1500, 375, 94, 23, and 6 copies of influenza A virus RNA were detected before and after lyophilization, respectively. Analysis was performed using a Bio-Rad CFX96 Touch Deep Well.

Good amplification was obtained in all cases and no freeze-drying effects were observed.

	Lyophilization	
	After	Before
PCR efficiency	95.0%	99.4%
r	-0.997	-0.997



Product Name	Size	Code No.
THUNDERBIRD™ Next Probe One-step qRT-PCR 4×Mix	1.25mL×1	QRX-101

It can be used for 250 times with the 20 µL reaction system and for 100 times with the 50µL reaction system.
50 × ROX Reference dye (100 µL) is attached separately and can be used for most real-time PCR analyzers.
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Storage temperature : -20℃

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