DESCRIPTION

TOROBlue® qRT Premix with gDNA Eraser enables fast cDNA synthesis for 2-step RT-qPCR and includes a rapid step that eliminates genomic DNA contamination without loss of input RNA. Genomic DNA is eliminated by treating the RNA templates with gDNA Eraser Mix for 2-5 minutes at 37°C. 5×qRT Premix contains primers and buffer optimized for highly efficient synthesis of short-chain cDNAs suitable for qPCR. The protocol is simple, and the reaction can be completed in 10-25 min.

COMPONENTS

The kit includes the following reagents,which can be used for 100 reactions for a total 10µl reaction volume.
Cat NO： RTQ-201

RNA Dilution Buffer	1ml/tube
gDNA Eraser Mix	200µl/tube
5×qRT Premix	200 µl/tube
RT Enzyme Mix	100µl /tube

Notes:

-RNA Dilution Buffer can be used to dilute and store RNA instead of RNase-free water.
- gDNA Eraser Mix contains Heat labile dsDNA Nuclease, RNase inhibitor, and reaction buffer.
- 5×qRT Premix contains oligo dT primer, random primer, and reaction buffer.
-RT Enzyme Mix contains the highly efficient reverse transcriptase and an RNase inhibitor.

PROTOCOL

1. Preparation of the reagent and templates

-This kit should be fully thawed before use. Gently vortexed and briefly centrifuged.
-Purified template RNA can be may be used directly or after dilution。

2. Genomic DNA elimination reaction

-Prepare the following reaction in a thin-walled PCR tube one the ice.

Component	Volume
gDNA Eraser Mix	2 µL
Total RNA	X µL
RNA Dilution Buffer	5-XµL
Total	7µL

-Incubate at 37℃ for 5 min. Store the reaction tube one ice.

Notes:

-The indicated time of gDNA elimination reaction is between 2-5min at 37℃.
-Up to 0.5 µg of total RNA template for qPCR assay.

3. Reverse-transcription

-Transfer 2 µL 5 × qRT Premix and 1 µL RT Enzyme Mix into the above reaction tube. Gently vortexed and briefly centrifuged.
-RT reaction as the following condition.

37℃ 15min
50℃ 5min
98℃ 5 min

-Store the reaction tube one ice.

Notes:

-The cDNA products can be used directly or after dilution for real-time PCR. Up to 20% of the synthesized cDNA solution can be added to the PCR reaction solution.
-The mutant M-MLV reverse transcriptase excels at high reaction temperatures (up to 60℃). This step may increase the efficiency of the reverse transcription.

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APPLICATION DATA

1. Efficiency of gDNA elimination.

RT Reagent: *TOROBlue® qRT Premix with gDNA Eraser (RTQ-201)*

Template: *Human Genomic DNA 50ng/reaction*

Experiment groups:

	4 × gDNA Eraser Mix	5 × qRT Premix	RT Enzyme Mix
A	(+)	(+)	(-)
B	(-)	(+)	(-)

qPCR Reagent: *TOROGreen®qPCR Master Mix (Code No.QST-100).*

Template: *cDNA 2 μL/20 μLreaction*

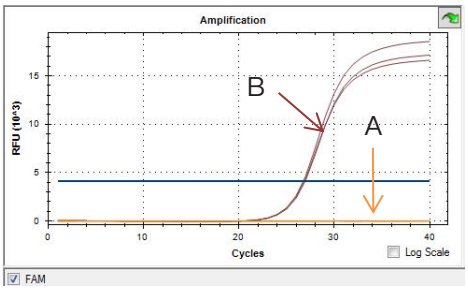
Target:*GAPDH (Non cross-intron)*

Forward primer：*AAAAGGGCCCTGACAACTCTT*

Reverse primer：*GTCTTACTCCTTGGAGGCCA*

Instrument ： *CFX-96 from Biorad*

Results：



No signal for the "A groups" indicates that 50ng gDNA was completely removed by gDNA Eraser.

2.Comparison of RT performance

RT Reagent: *TOROIVD ® qRT Master Mix(RTQ-100).*

TOROBlue® qRT Premix with gDNA Eraser (RTQ-201)

Template: Template RNA:*MS2RNA (Roche)*

Forward primer: *GCCTTAGCAGTGCCCTGTCT*

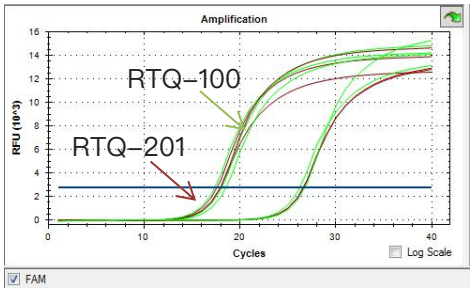
Reverse primer:*AACATGCTCGAGGGCCTTA*

qPCR Reagent: *TOROGreen®qPCR Master Mix (Code No.QST-100).*

Template: *cDNA 2 μL/20 μLreaction*

Instrument ： *CFX-96 from Biorad*

Results：



Despite the gDNAcontamination, the results of RTQ-201 correlate highly with those of the RTQ-100. Both reagents showed highly linear standard curves in a broad concentration range.

STORAGE

This reagent should be kept at -20°C for 1 years。