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# TOROBlue® RT Kit for qPCR-FD-

**【Catalogue Number】** RTQ-108

**【Packing Information】** 120 reactions for a total 20μL reaction volume

(or 240 reactions for a total 10μL reaction volume )

**【Description】**

TOROBlue® RT Kit for qPCR-FD- is a room-temperature stable RT kit to perform for qPCR.The engineered reverse transcriptase in this kit allows the synthesis of high-quality first-strand cDNA. 2×RT buffer for qPCR is 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.

**【Feature】**

**-Room-Temperature stable:** the performance is not easily decrease during during storing and shipping.
**-Easy-to-use:** add 50μL 2×RT buffer into a single tube to prepare the RT premix easily.
**-Maximum flexibility:** cutting each tube allows for 5 or 10 RT reactions.
**-High performance：** an engineered reverse transcriptase allows the synthesis of high-quality cDNA.

**【Components】**

TOROBlue® RT Kit for qPCR-FD- includes the following reagents,which can be used for 120 reactions for a total 20μL reaction volume or 240 reactions for a total 10μL reaction volume .

| NO. | Components | Size |
|---|---|---|
| 1 | Freeze-dried RT PreMix-002- | 8-tube strips×3 |
| 2 | 2×RT buffer for qPCR | 1.25mL/tube×1 |
| 3 | Water-DEPC treated | 1.25mL/tube×1 |

**Notes:**

*-Freeze-dried RT PreMix-002 contains lyophilized Reverse transcriptase, RNase Inhibitor and , Random hexamer and/or oligo(dT)20 primers;2×RT buffer for qPCR contains reaction buffer optimized for highly efficient synthesis of short-chain cDNAs suitable for qPCR.*

**【Protocol】**

**1. Preparation of the 2× RT premix**

-Open the reagent packaging and check if the freeze-dried powder in the tube is deliquescent.
***Notes:*** *It is not recommended to use moisture absorbing test tube.*
-Cut open the 8-strips tube according to the required quantity.
***Notes****: The remaining test tubes should still be sealed in a dry environment.*
-Add 50 μL 2×RT buffer into each tube to prepare the 2× RT premix.
-Mix gently with a pipette and briefly centrifuged.

**2. Preparation of RNA template.**

-Use the purified RNA templates after quality assessment and genomic-DNA contamination assessment according to the MIQE guidelines.
-Instead of using 2× RT premix, 2×RT buffer as no-reverse transcription control was used to assessed the extent of genomic-DNA contamination in the following steps.
***Notes:****If gDNA contamination affects the Cq values, it is is essential to eliminate it by DNase treatment.*
-RNA diluent should be prepared in a thin-walled PCR tube as follows.

| Component | Volume |
|---|---|
| Water-DEPC treated | 10-XμL |
| Total RNA(up to 3μg) | X μL |
| Total | 10μL |

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**3. Preparation of the reaction mix.**

-Prepare the following reaction mix in the above reaction tube with RNA diluent.

| Component | Volume |
|---|---|
| RNA diluent ( **Step 2)** | 10 μL |
| 2× RT premix ( **Step 1)** | 10 μL |
| Total | 20 μL |

-Mix gently with a pipette and briefly centrifuged.

**4. RT reaction Condition**

37℃, 15min
50℃, 5min
98℃, 2 min

-Store the reaction tube one ice.
-Transfer 100μL RNase free water into the above reaction tube.
-Gently vortexed and briefly centrifuged.
-The cDNA products can be used directly or after dilution for real-time PCR.

**【Storage】**

Store the test tube at 2-8℃ in a dry environment for 24 months.

**【References】**

Bustin SA, Benes V, Garson JA, etc,al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments.ClinChem.2009,Apr;55(4):611-22.

**【Application】**

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**【Contact】**

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