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TOROIVD® RTase Screening Kit

【Catalogue Number】 RRM-804FD

【Packing Information】 8-strips tube ×4 types

【Description】

TOROIVD® RTase Screening Kit, cotainning 4 kind of freeze-dried premix, is specially designed for IVD reagent manufacturers to screening the optimal raw materials. The freeze-dried RT premix contains the 4 kind of reverse transcriptase and RNase Inhibitor in 8-strips tube. The 4 kinds of RTase was widely used as raw materials for IVD reagent manufacturers and followed the ISO13485 quality management system for large-scale production.

【Feature】

-Shorten R&D cycle

No need to optimize the buffer, the optimal RTase for 1-Step RT-qPCR can be quickly screened.

-Room-temperature stable:

The performance is not easily decrease during storing and shipping.

-Easy-to-use:

Add 100μL 2×qPCR Premix into a single tube to prepare 1-Step RT-qPCR premix easily.

-High performance：

Four kind of engineered reverse transcriptase to be compatible with different PCR buffer allows 2-copies targets per reaction can be detected.

【Application】

Development of IVD reagents based RT-qPCR

【Components】

Components (Name/Cat No.)	Size	Quantity
Freeze-dried RI& RTase PreMix-002-(RRM-002FD)	8-tube strips	1
Freeze-dried RI& RTase PreMix-003-(RRM-003FD)	8-tube strips	1
Freeze-dried RI& RTase PreMix-005-(RRM-005FD)	8-tube strips	1
Freeze-dried RI& RTase PreMix-007-(RRM-007FD)	8-tube strips	1

【Primer/Probe Design】

-Design of primers

Primer length: 18–25bp； Tm of primer: 60–65°C； GC content: 40–60%； Target length: 70–200 bp;
Purification grade: OPC or HPLC grade;

-Design of probes

Probe length: 20–30bp; Tm of probe: 65–70°C; GC content: 40–60%; Purification grade: HPLC.

-Design for avoiding Contamination

Amplicon with high GC content will degrade the the performance of UNG for removing the contamination caused by PCR product.Therefore, when designing the primers, it is necessary to minimize the GC content of the amplicon as much as possible.

【Template Preparation】

1. Purified template RNA can be may be used directly or after dilution
2. For direct 1-Step RT-qPCR with extraction-free samples, the premix may be inhibited by some VTM or dilution buffer. It is recommended the VTM or dilution buffer based Tris buffer, such as TE buffer. The addition of surfactants (Triton X-100 or Tween-20)within 5% helps to lysis the sample and inactivate viruses.

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3. Loading 10μL purified template or diluted sample per well in two 8-strip qPCR tubes.

Notes: *It is recommended to use approximately 100copies targets per reaction*

【Protocol】

1. Preparation of the 1-step RT-qPCR 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 100μL 2× qPCR premix into each tube to prepare the 1-step RT-qPCR Premix.
- Mix gently with a pipette and briefly centrifuged.

Notes:*The 2× qPCR premixes should be fully thawed , gently vortexed and briefly centrifuged.*

2. Preparation of the reaction mix

-Prepare the primers and probes for 4×primer&probe premix

Note: *The final concentration is recommended to 0.4 μM for the primes and 0.1 μM for the probes.*

-Prepare the following reaction mix for 5 reactions at total 40μL reaction volume as follows :

Component	5 reactions
2×1-step RT-qPCR Premix	100μL
4×Primer&Probe Premix	50μL
Total	150 μL

-Mix thoroughly by vortexing and centrifuge immediately.

-Loading 30 μL reaction mix per well in the qPCR tube with 10μL template.

-Set four technical replicates for each premix.

-Gently vortexed and briefly centrifuged.

3. Set up the qPCR cycler:

-Select the corresponding fluorescence detection channel based on the fluorescence labeling of the probe.

-Set up the universal cycling conditions for all qPCR cyclers as follows:

Steps		Temperature	Time	Cycles
1	UNG enzyme action	37°C	2 min	1
2	Reverse transcription	50°C	10min	1
2	Prenaturation	95°C	3min	1
3	Denaturation	95°C	15 sec	45
	Annealing/ Extension	60°C	30 sec	
Data collection should be performed at the extension step.				

4. Result analysis

-Select the Select the optimal RI& RTase PreMix based on the Cq value and fluorescence signal value.

-Contact us to order the optimal freeze-dried RI& RTase PreMix for further development and optimization.

【Storage】

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

【Contact】

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