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FAST ONE STEP PATH TAQMAN PROBE RT- qPCR Multiplex FS-RT-41320

Description

FAST One Step Path TAQMAN PROBE RT-qPCR Master Mix is a ready-to-use kit Multiplexing, allowing Fast-Cycle Amplification,One Step reverse transcription qPCR in a single tube on pathogen and viral samples.It is supplied with a separate vial of ROX Dye, and contains all components for RT-qPCR except primers, probes RNA templates. The one-step format significantly improves sensitivity and effectively prevent contamination. The heat-liable dUTP/UDG prevents contamination and degrades uracyl-contaminants at RT, to avoid false positive results. At 50°C during reverse transcription the heat-labile UDG quickly deactivates ensuring the efficiency and sensitivity of RT-qPCR. The Script Reverse Transcriptase in the kit provides reliable reverse transcription to a wide range of RNA template amount.

Kit Contents:

FS-RT-41320 – 100 reactions	Size 100 rns
Fast One Step Path Taqman Probe RT-qPCR Enzyme Mix **	80 µl
Fast One Step Path Taqman Probe RT-qPCR Buffer *	320 µl
50X ROX Dye I (High Rox)***	1 vial
50X ROX Dye II (Low Rox)***	1 vial

** the Master Mix is blocked by antibody, containing RNase Inhibitor, Heat-labile UDG

* Containing dNTP/dUTP Mix, prevent false positive caused by cross contamination with UDG.

*** Passive reference dye to normalize the fluorescence signals

Applications

- Real-time qPCR – Multiplexing Fast Cycles
- Detection and quantification of DNA and RNA targets

Storage Conditions

Upon receipt, store all components at –20°C.

Use of the ROX Reference Dye:

-50x Rox Dye I (High Rox)

Applied Biosystems 7000/7300/7700/7900, Applied Biosystems StepOne™/StepOnePlus™.

-50x ROX Dye II (Low Rox)

Applied Biosystems 7500/ViiA7TM, QuantStudio™, Stratagene Real-time PCR Systems, Rotor-gene™ 3000

-NO ROX Dye

Bio-Rad iCyclers/ CFX96/ CFX 384, Roche Light Cyclers®, QIAGEN/Corbett Systems, Eppendor Mastercyclers

Recommended Protocol

1. Fully thaw the Fast One Step Path Taqman Probe RT-qPCR Buffer, and the Master Mix before use.

2. The FAST One Step Path Taqman Probe RT-qPCR Enzyme Mix contains high concentration of glycerin. Mix gently before use without generating air bubbles. Spin briefly to collect all the contents at the bottom. When preparing multiplexing reactions simultaneously, prepare a mixture of all the reagents and aliquote into each reaction tube, to avoid loss. After use, return it to -20°C immediately.

3. If applicable, use aerosol-resistant pipette tips and microtubes to minimize contamination.

4. High quality RNA templates are recommended for optimal results

5. Only gene specific primers are recommended.
Random primers and Oligo dT primers are NOT recommended in the reverse transcription reaction.

6. The optimal length of amplicon is between 70 and 200 bp for general cycling conditions.

Prepare materials before reaction setup:

Pipette, aerosol-resistant pipette tip, cold blocks and ice.

Gene expression primers and probes.

RNA templates.

1.5 mL RNase-free EP tubes, Real-time PCR tubes

and plates.

1. **Prepare the reaction mix** :

Set up the reaction on ice by adding the following components for the number of reactions required. :

Reaction Conditions

Component	20 µL reaction
Fast One Step Path Taqman Probe RT-qPCR Enzyme Mix	0.8 µl
Fast One Step Path Taqman Probe RT-qPCR Buffer	3,2 µl
10uM Forward Primer*	X
10uM Reverse Primer*	X
Probe (10µM)***	X
50X Rox Dye (optional)	0.4 µl
Total RNA **	2 µL
Water, RNase-Free	Up to 20µl

* A final primer concentration of 0.2 µM is recommended for most reactions. However, to optimize individual reaction, a primer titration from 0.1 µM to 1.0 µM can be performed. The length of amplified PCR products should ideally be in the range of 70 - 200bp.

** Use 10 pg~100 ng of RNA template in a 20 µL reaction.

*** A Probe concentration of 50-250 nM is recommended.

***Note: 1)** The amount of primers/probe needs to be titrated for the desired concentration for fast programs, which may differ from standard reactions .

The optimal concentration range for primers and probes in the FAM channel is 0.16-0.32 µM, and for the VIC/ROX/TAMARA channels, the concentration range is 0.32-0.48 µM.

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2）For reaction volumes of 30 µL and 50 µL,amplification can be carried out on machines such as ABI StepOne Plus, ABI 7500,and Bio-rad CFX-96 according to the corresponding proportions.

Optimized One Step RT-qPCR Conditions

Step	Temp (°C)	Time	Cycle
Reverse Transcription	50°C	2 min.	1
Predenaturation	95°C	2 sec.	1
Cycles reaction	95° C	10 sec.	41
	60° C	5-10 sec.	

The extension time should be adjusted to the minimum time required for data acquisition according to qPCR instrument guidelines used. (30 seconds for ABI Step One Plus 31 seconds for ABI7300, and 34 seconds for ABI 7500)

The extension time for other instruments needs to be tested according to the instrument's specifications. Note: This product is also compatible with the conventional amplification program: Reverse Transcription at 50°C for 5 min, Pre-denaturation at 95°C for 3 min, Denaturation at 95°C for 15 seconds, and Extension at 60°C for 30 seconds.

3. After the reaction, confirm the Real-Time PCR amplification curve and proceed with standard curve construction

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