Research Use Only

Product Name and Catalog Number

1-Step 2X RT-PCR Master Mix-TaqMan Probe Cat. No. W143

Intended Use

- The 1-Step 2X RT-PCR Master Mix is used for qualitative and quantitative RT-PCR amplifications with TaqMan probe.
- The master mix is a premixed, 2X concentrated solution that has all the components except for gene-specific primers, probe and RNA template.

Kit Characterizations

- For the reverse transcription step, this kit uses a highly efficient Thermophilic Reverse Transcriptase (US patent pending), which is a thermophilic type A polymerase with optimal temperatures of 60-62°C, and it can be heat-inactivated at ≥90°C.
- The thermophilic enzyme highly efficiently synthesizes a complementary DNA strand on RNA template, *one unit per 20μL of reaction.*
- The kit can detect single digit copies of target RNA depending on assay design and optimization.
- The concentrations of the primers and probe are variable depending on specific assays and thermo-cycling protocols (Table 1).
- In the 1x reaction mix, 3mM $MgCl_2$ is preset.
- The preferable PCR product size is ≤150bp.
- **No ROX passive reference dye** is in the master mix.

Kit Contents

2X Master Mix (1000 μL) for 100 or 200 RT-PCR reactions and an instruction for use.

Transportation and storage

The kit can be transported at ≤4°C for up to 3 days. The kit should be stored in the dark at -20°C for 12 months without more than 10 times of freeze-thaw cycles. The kit can be stored at ≤4°C for a shot time.

Table 1. Setting up a 20 μL or 10 μL reaction

Component	Volume per 20μL	Volume per 10μL	Final concentration
2X Master Mix	10μL	5μL	1X
Primers[a]	Variable	Variable	Each 150-900nM
TaqMan probe[b]	Variable	Variable	150-250nM
RNA template[c]	Variable	Variable	As low as single digit copies of target RNA to ≤1μg total RNA
H_2O	To 20μL	To 10μL	

[a]The primer's T_m should be designed ≥60°C using primer3 software for high efficiency at the optimal temperature.

[b]The probe's T_m should be 8-10oC higher than the primer's T_m.

[c]RNA templates should be extracted by a qualified silica-based kit and eluted with low EDTA TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0-8.3).

- After setting up the reactions, seal the 96-well plate/8-tube strip(s).
- Vortex to fully mix the components, and centrifuge shortly to remove air bubbles.
- Then put the 96-well plate/8-tube strip(s) into the real-time fluorescence PCR instrument.
- Note: Preferably operate on ice. Avoid prolonged exposure to intense light to avoid bleaching the fluorescence.

Applicable Instruments

This kit is compatible with Bio-Rad real-time PCR detection systems, Applied Biosystems/ThermoFisher real-time PCR instruments, Roche LightCycler LC480, QIAGEN Rotor-Gene Q, Eppendorf Mastercycler EP realplex, and Stratagene Mx real-time PCR systems.

Note: **Turn off ROX passive reference dye button** when setup assays on Applied Biosystems/ThermoFisher instruments.

Setting Up Thermal Cycling

Table 2. Standard Thermo-Cycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10 min	1
II	95°C	2 min	1
III	95°C	12 sec	35-40
	60°C, signal acquisition	60 sec	

Table 3. Fast Thermo-Cycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10 min	1
II	95°C	1 min	1
III	95°C	10 sec	35-40
	60°C, signal acquisition	30 sec	

Quality control

Not detectable DNase and RNase activities.

Related Products

- Thermophilic Reverse Transcriptase, Cat. No. W140
- 1-Step 2X RT-PCR Master Mix-SYBR Green-**HY** (High Yield), Cat. No. W141
- 1-Step 2X RT-PCR Master Mix-SYBR Green-**HP** (High Processibility), Cat. No. W142