Research use only

1-Step 2X RT-PCR Master Mix-SYBR Green Dye

Product Name and Catalog Number

1-Step 2X RT-PCR Master Mix-SYBR Green Dye, Cat RT-PCR 144, 2x1mL/5x1mL/10mL for 200/500/1000 reactions x 20μL (No ROX, Low ROX or High ROX)

Intended Use

- The 1-Step Fast 2X RT-PCR Master Mix is used for real-time qualitative and quantitative RT-PCR amplifications with SYBR Green dye particularly for fast thermocycling.
- The master mix is a premixed, 2X concentrated solution that has all the components except for gene-specific primers and RNA template.

Kit Characteristics

- The kit is designed for singleplex RT-PCR with SYBR Green dye.
- 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.
- The RTase is easily heat-inactivated at ≥90°C for 1min.
- The RTase efficiently synthesizes a complementary DNA strand on RNA template from a gene-specific primer, *≤1 unit per 20μL of reaction.*
- The RTase reversely-transcribes single digit copies of target RNA molecules consistently.
- The kit also contains *Taq* DNA polymerase for PCR with SYBR Green dye.
- The concentrations of the primers are variable depending on assay designs and thermocycling protocols (Table 1).
- The preferable PCR product size is ≤150bp.
- The kit has three formulations of ROX, Low ROX or High ROX concentrations for your choice.

Kit Contents

2X Master Mix (2x1mL/5x1mL/10mL for 200/500/1000 reactions x 20μL) and an instruction for use.

Transportation and Storage

The kit can be transported at below 4°C for up to 3 days.

The kit should be kept stable in the dark at -20°C for ≤24 months with ≤10 times of freeze-thaw cycles. The kit can be stored at 4°C for a weak.

Setup Reaction and Thermocycling

1. Thaw 1-Step 2X RT-PCR Master Mix and other reaction components at room temperature, mix each component, centrifuge and then place on ice.

2. Determine the total volume for the number of reactions, add 5-10% extra volume, and prepare assay mix of all components except RNA template. Mix the assay mix, centrifuge and then place on ice.

3. Aliquot the assay mix into PCR tubes or plate.

4. Add RNA template to PCR tubes or plate.

5. Seal tubes with flat, optically transparent caps or seal plates with optically transparent film.

6. Mix and then briefly centrifuge the tubes or plate.

7. Program PCR instrument with indicated thermo-cycling protocol.

8. Load PCR tubes or plate and start to run.

9. Perform data analysis according to the PCR instrument instructions.

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

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

Footnotes of Table 1

[a]The primer's T_m should be designed ≥60°C, preferably between 62°C to 65°C, using primer3 software for high efficiency and specificity.

[b]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).

Table 2. Compatible instruments

RT-PCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™	Not recommended	Not necessary

Eppendorf Mastercycler® Cepheid® SmartCycler®		
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye button
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye button

Table 3. Standard thermocycling protocol

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

Table 4. Three-Step Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal acquisition	30sec	
IV	68°C to 95°C	Various	1

Footnotes of Tables 3 and 4

The three-step thermocycling protocol in Table 4 increases overall DNA polymerase activity by 50%, a more effective protocol than Table 3.

The primer concentration used in Tables 3 and 4 is typically 0.15-0.2uM.

Related Products

- Thermophilic Reverse Transcriptase, Cat RT-PCR 140
- 1-Step 2X RT-PCR Master Mix-TaqMan probe, Cat RT-PCR 143
- *Taq*-Probe Polymerase, Cat RT-PCR 145
- 1-Step 2X Multiplex RT-PCR Master Mix-TaqMan probe, Cat RT-PCR 146
- 1-Step 2X Fast RT-PCR Master Mix-SYBR Green, Cat RT-PCR 147
- *Taq*-Fast Polymerase, Cat RT-PCR 148
- 1-Step 2X Super Multiplex RT-PCR Master Mix-TaqMan Probe**,** Cat RT-PCR 149

Precautions

If you order a "**No ROX**" master mix but have an Applied Biosystems/ThermoFisher instrument, please **turn off ROX passive reference dye button** when setup assays.