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

Product Name and Catalog Number

1-Step 2X Fast RT-PCR Master Mix-SYBR Green, Cat. W147-NR, W147-LR, W147-HR, 2x1mL for 200 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*-Fast DNA polymerase which extends more than 300 bases with short cycling program.
- The concentrations of the primers are variable depending on assay designs and thermocycling protocols (Table 1).
- The preferred 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 for 200 reactions x 20µL)

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 week.

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 plates.
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 plates 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.

Table 5. Fast thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	60°C	5-10min	1
II	95°C	1min	1
III	95°C	5sec	35-40
	60°C, signal acquisition	30sec	
IV	60°C to 95°C	Various	1

Footnotes of Table 5

The product size for the fast-thermocycling protocol is preferred to be less than 90bp.

The primer concentration used is typically between 0.4uM and 0.9uM.

Related Products

- Thermophilic Reverse Transcriptase, Cat. W140
- *Taq*-Probe Polymerase, Cat. W145
- *Taq*-Fast Polymerase, Cat. W148
- 1-Step 2X Fast RT-PCR Master Mix-SYBR Green, Cat. W147
- 1-Step 2X RT-PCR Master Mix-TaqMan probe, Cat. W143
- 1-Step Multiplex 2X RT-PCR Master Mix-TaqMan probe, Cat. W146
- 1-Step 2X Super Multiplex RT-PCR Master Mix-TaqMan Probe, Cat. W149

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.