1-Step RT-qPCR Supermix (Probe-based)

Catalog No.:2306-001 Size: 1 ml
Catalog No.:2306-005 Size: 5 ml

Storage:

−25°C to −15°C

Component	2306-001	2306-005
1-Step RT-qPCR Supermix (Probe-based)	1 ml	5 x 1 ml

Description

1-Step RT-qPCR Supermix (Probe-based) is specifically designed for rapid and efficient real-time reverse transcription quantitative PCR (RT-qPCR). This premix can be used with TaqMan™ or other probe-based assays for both DNA and RNA targets.

The premix contains optimized M-MLV reverse transcriptase and hot-start DNA polymerase, ensuring superior sensitivity and performance throughout the thermal cycling process. With 1-Step RT-qPCR Supermix (Probe-based), you can achieve accurate results in a shorter time, thereby improving experimental efficiency.

Preparation of RT-qPCR Assay

1. Thawing Gene Expression Assays
   - For TaqMan™ probe sets or other Probe-primer sets, ensure a final concentration of 20X.

     Recommended final concentrations:
     - Primer: 400–900 nM
     - Probe: 100–250 nM
2. Dilution of 60X Assays to 20X
   - Dilute 60X stock solution to 20X working solution with TE buffer (pH 8.0).
   - Aliquot the diluted solution to minimize freeze–thaw cycles.

Component	**Volume**
Probe-Primer Set (60X)	40 µL
TE Buffer (pH 8.0, 1X)	80 µL
Total	120 µL

Store aliquots at −20°C until use.

Reaction Setup

1. Thaw all reagents on ice, mix thoroughly, spin briefly, and keep on ice.
2. Prepare the reaction mix as follows (20 µL or 50 µL final volume):

Component	**20 µl rxn**	**50 µl rxn**
1-Step Supermix	5 µL	12.5 µL
Probe-Primer Set (20X)	1 µL	2.5 µL
DNA/RNA Sample	x µL (≤14 µL)	y µL (≤35 µL)
RT-PCR Grade Water	(14 − x) µL	(35 − y) µL

Seal reaction plates with optical film and spin briefly.

Thermal Cycling Conditions

- Fast Cycling Protocol

Step	**Temperature**	**Time**	**Cycles**
Reverse Transcription	50°C	5 min	1
RT Inactivation/Denaturation	95°C	20 sec	1
Denaturation	95°C	3 sec	40
Annealing/Extension	60°C	30 sec	40*

*Read fluorescence

- Standard Cycling Protocol

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	5 min	1
RT Inactivation/Denaturation	95°C	20 sec	1
Denaturation	95°C	15 sec	40
Annealing/Extension	60°C	60 sec	40*

*Read fluorescence

Note: Reverse transcription temperature can be adjusted between 37° C and 55°C.

Data Analysis

Refer to the instrument software for analysis using absolute or relative quantification (ΔΔCt) methods. Typical analysis steps include:

- Review amplification curves; adjust baseline and threshold if necessary.
- Exclude outlier wells if needed.
- Review Ct values for all wells and replicates.
- Good Laboratory Practice for qPCR
- Always wear clean gloves and lab coat; change gloves frequently.
- Separate areas and equipment for:
- Sample preparation and reagent handling
- Amplification and analysis
- Avoid introducing amplified products into pre-PCR areas.
- Keep reaction tubes closed whenever possible.
- Use filter tips and dedicated pipettes for different steps.
- Decontaminate work surfaces and equipment regularly with 10%bleach or DNA decontamination solutions.
- Do not open tubes after amplification.