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

Catalog No.: 2305-001 Size: 1 ml
Catalog No.: 2305-005 Size: 5 ml

Storage:

Ship with ice packs.
Store at 2–8°C for short-term use; Long-term storage at −25°C to −15°C.
After first use, store at 4°C protected from light and avoid repeated freeze–thaw cycles

Components	2305-001	2305-005
1-Step RT-qPCR Supermix (Dye-based)	1 ml	5 x 1 ml

Product Description

1-Step RT-qPCR Supermix (Dye-based) is a 2× dye-based premix designed for rapid and efficient real-time reverse transcription quantitative PCR (RT-qPCR) starting directly from RNA templates. This one-step formulation enables fluorescent quantification using a dye-based detection system.

The premix contains:

- dsDNA-binding dye (SYBR GREEN I)
- Optimized M-MLV reverse transcriptase
- Hot-start Taq DNA polymerase
- Heat-labile Uracil-N-Glycosylase (UNG)
- dNTPs (with dUTP)
- ROX reference dye
- Optimized buffer components

With 1-Step RT-qPCR Supermix (Dye-based), researchers can expect faster and more accurate results, improving experimental efficiency.

Reaction Setup

1. Thoroughly mix 1-Step RT-qPCR Supermix (Dye-based).
2. Thaw RNA samples and primers on ice, vortex gently, spin briefly, and keep on ice.
3. Prepare reactions according to the table below. Allow 10% extra volume to compensate for pipetting losses when preparing multiple wells.

Component	20 µL Rxn	10 µL Rxn	Final Conc.
1-Step RT-qPCR Supermix (Dye-based)	10.0 µL	5.0 µL	1X
20 µM Forward Primer	0.5 µL	0.25 µL	500 nM*
20 µM Reverse Primer	0.5 µL	0.25 µL	500 nM*
RNA Sample	≤9 µL	≤4.5 µL	Adjust as needed
RT-PCR Grade Water	to 20 uL	To 10 µL	To final volume

*Primer concentrations may be adjusted between 50–900 nM for optimal performance. Recommended RNA input: 1 pg–1 µg total RNA.

4. Seal the reaction plate with optical film and press firmly.
5. Briefly centrifuge to eliminate air bubbles.

RT-qPCR Thermal Cycling Conditions

Follow instrument manufacturer's instructions for thermal cycling settings and compatible consumables. The premix supports both fast and standard cycling modes.

a) Fast Cycling Program

Step	Temp.	Time	Cycles
Reverse Transcription	37°C	15 min	1
High-Temp RT*	50°C	5 min	1
RT Inactivation/Denaturation	95°C	5 min	1
Denaturation	95°C	20 sec	40
Annealing/Extension	60°C	30 sec	40*

*Read fluorescence

b) Standard Cycling Program

Step	Temp.	Time	Cycles
Reverse Transcription	37°C	15 min	1
High-Temp RT*	50°C	5 min	1
RT Inactivation/Denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	40
Annealing/Extension	60°C	60 sec	40*

*Read fluorescence

Melting Curve Analysis*:

95°C 15 sec → 60°C 15 sec → 95°C 15 sec

* High-temperature RT helps resolve complex RNA secondary structures, improving RT efficiency for difficult RNA templates. Melting curves verify amplification specificity; use instrument default settings if available.

Data Analysis

1. Examine amplification curves; adjust baseline and threshold settings as needed.
2. Check melting curves for signs of non-specific amplification.
3. For absolute quantification, evaluate:
    - Standard curve slope
    - Amplification efficiency
    - R^2 value
    - Y-intercept
    - Ct values
    - Outliers