PowerScript RT SuperMix

For research use only

Components

Component	R1081 (100 rxns)	R1082 (500 rxns)	R1083 (2500 rxns)
PowerScript RT SuperMix	400 μL	400 μL × 5	10 mL

Storage

This reagent should be kept at -20°C and is valid for 2 years.

Description

PowerScript RT SuperMix is an optimized 5X reverse transcription mixture that contains all the ingredients required for first strand cDNA synthesis in a two-step RT-qPCR workflow. It contains thermostable reverse transcriptase, which supports cDNA synthesis at high temperatures. It also contains murine-derived RNase inhibitors to protect the template RNA from degradation. The mixture contains random haxmers and Oligo dT primers, which can fully perform reverse transcription of various kinds of RNA. This product can reverse transcribe as low as a single-copy of RNA, and the resulting cDNA product is an ideal template for qPCR detection, ensuring the sensitivity and accuracy of detection.

Application

First strand cDNA synthesis.

Matters Needing Attention

- Successful cDNA synthesis comes from high quality RNA. High quality RNA should at least guarantee full length integrity and contain no inhibitors of reverse transcriptase, such as EDTA or SDS.
- The amount of RNA required for detection depends on the abundance of the transcript of interest. In general, the recommended total RNA is 1 ng to 1 μg or mRNA is 0.1 ng to 100 μg.
- During the operation, care should be taken to prevent RNA degradation due to the introduction of RNase. It is best to store RNA at -70°C and avoid repeated freezing and thawing.
- The presence of genomic DNA or excess product can interfere with the accurate quantification of target RNA, especially for low-copy targets. The No RT control reaction can be simulated by thermal inactivation of PowerScript RT SuperMix at 95°C for 1 minute and then adding template RNA. In addition, an NTC (no template control) reaction should be set up to demonstrate that the reverse transcription is meaningful.
- cDNA products should be stored at or below -20°C and avoid repeated freeze-thaw.

Protocol

1. Prepare the reaction system

Gently mix the product and prepare the reaction mixture in an RNase-free centrifuge tube according to the following system:

Component	Amount	Final Conc.
PowerScript RT SuperMix	4 μL	1X
RNA sample	1 pg-1 μg*	-
RNase-free ddH_2O	to 20 μL	-

* The maximum amount of RNA supported by the 20 μL reaction system is: 1 μg total RNA, or 1 μg mRNA, or 100 ng specific RNA. If the amount of RNA needs to be increased, the reaction system needs to be increased accordingly to ensure the linearity of subsequent quantitative detection results.

NTC reaction (optional)

Prepare the reaction mixture in an RNAse-free centrifuge tube according to the following system:

Component	Amount	Final Conc.
PowerScript RT SuperMix	4 μL	1X
RNase-free ddH_2O	16 μL	-

2. RT reaction

Blow and mix the above mixture with pipette, then collect by instantaneous centrifugation to the bottom of the tube.

Run the reaction in the PCR instrument according to the following procedure:

Step	Temperature	Time	Cycle
Primer annealing	25°C	2 min	1
cDNA synthesis	55°C*	10 min	
Heat inactivation	95°C	1 min	

* Reverse transcription reactions can be performed at 45 ° C ~65 ° C, with 55 ° C being the optimal temperature for most reactions.

3. qPCR reaction and cDNA preservation

The cDNA product can be diluted and immediately subjected to qPCR by dye method or probe method. If undiluted cDNA stock solution is used, the volume should not exceed 20% of the reaction system to avoid inhibiting the reaction efficiency. Long-term storage of cDNA products should be in -20°C or below environment, and avoid repeated freeze-thaw.