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One Step SYBR Green RT-PCR Mix

Cat. No	Specification	Storage/Shelf life
EQ007-01	20μL x 100 rxns	-20C°/2 years
EQ007-02	20μL x 500 rxns	-20C°/2 years

Introduction

This product is a special reagent for Real Time One Step RT-PCR using the SYBR Green method. Using this product for Real Time RT-PCR reaction can continuously perform reverse transcription and PCR amplification in the same reaction tube. It is simple to operate and can effectively prevent contamination. Since this reaction system can monitor the amplified products in real time, the detection sensitivity is greatly improved, and the electrophoresis step after PCR reaction is omitted, which is very suitable for the detection of RNA viruses.

This product uses high-efficiency reverse transcriptase and high-specificity hot-start Taq DNA polymerase to perform stable and efficient Real Time One Step RT-PCR reactions. For the fluorescent quantitative PCR instrument that uses ROX as the calibration dye, this product is equipped with a separate ROX dye to correct the fluorescent signal error generated between the wells of the quantitative PCR instrument.

Kit Components

Components	EQ007-01	EQ007-02
2×One Step RT-PCR Mix（SYBR Green）	1ml	1.25 ml×4
RT-PCR Enzyme Mix	150 μl	750 μl
50×ROX Dye	250 μl	1.25 ml
RNase-free ddH2O	1 ml	1.25 ml×4
User manual	1 copy	1 copy

Reagents and items that users need to prepare

1. PCR primers.
2. RNA template.
3. Suitable for single tube, 8-strip tube, or 96-well PCR tube (plate) for fluorescent quantitative PCR.
4. Micropipette and clean tip with filter element.
5. Real Time PCR Thermal Cycler.

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Note

1. Before use, please turn it upside down and mix gently, try to avoid foaming, and use it after a short centrifugation.
(1). Do not mix by vortex shocking.
(2). Since the enzyme preservation solution contains 50% glycerin and has a high viscosity, it should be absorbed slowly when dispensing.
(3). Precipitation will result in uneven composition of the solution. Be sure to mix the reagents thoroughly before use.
2. Avoid strong light when storing this product or preparing PCR reaction solution.
3. Minimize the number of repeated freezing and thawing of products, as repeated freezing and thawing may reduce product performance.
4. When preparing the reaction solution, please use clean tips (it is recommended to use tips with filter) and centrifuge tubes to prevent contamination as much as possible.
5. When preparing the reaction solution, keep the reagents on ice.

Instructions (recommended reaction system)

1. Prepare PCR reaction solution according to the following components and place on ice.
2. Turn the thawed components upside down and mix them evenly, and add each group to the following table to make a PCR reaction system:

Composition	Volume（μL）		Final Concentration
2×One Step RT-PCR Mix	10	25	1 x
RT-PCR Enzyme Mix	1.5	3.75	
PCR Forward Primer (10 μM)	0.4	1	0.2μM
PCR Reverse Primer (10 μM)	0.4	1	0.2μM
Templated RNA	Total RNA 1 pg-1 μg		
*50 x ROX Dye (optional)	0.4	1	1x
RNase-free ddH2O	to 20	to 50	——

Note: 1) The length of the amplified product should be within the range of 80-250bp.

2) 20 μL or 50 μL is recommended for the reaction system.

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***ROX Dye**

ROX dye can be added to the reaction system according to the selected instrument to standardize the fluorescence signal in the reaction system. The following table lists the amount of ROX (per 50μL reaction system) required when operating with different instruments:

Instrument	The amount of ROX required for each 50μL system reaction
ABI7300、7900HT、StepOne etc.	5μL
ABI7500、7500Fast、 ViiA7、Stratagene Mx3000™、Mx3005P™ and Mx4000™ etc.	1μL
Roche、Bio-Rad，Eppendorf etc.	/

Note: Please use the final concentration of 0.2 μM-0.6 μM as the reference for the setting range of the final primer concentration. When the amplification efficiency is not high, the primer concentration can be adjusted within the range of 0.1~1.0 μM

Two-step amplification procedure:

Steps	Cycle number	Temperature	Time
reverse transcription	1x	50℃	15 min
Pre-denaturation	1x	95℃	2 min
Denaturation	35-40x	95℃	10 sec
Annealing/ Extended		60℃	30 sec
Melt Curve			

Three-step amplification procedure:

Steps	Cycle number	Temperature	Time
reverse transcription	1x	50℃	15min
Pre-denaturation	1x	95℃	2 min
Denaturation	35-40x	95℃	10 sec
Annealing		50~60℃	30 sec
Extended		72℃	30 sec
Melt Curve			

Note: The annealing temperature and time can be adjusted according to the length of the primer and the target gene.

Result analysis

At least three biological replicates are required for quantitative experiments. After the reaction is over, it is necessary to confirm the amplification curve and melting curve.

Choice of experimental conditions

When selecting experimental conditions, please consider comprehensively from the two aspects of amplification specificity and amplification efficiency. A reaction system that can meet these two conditions at the same time is required to perform good quantification in a larger concentration range.
An experimental system with high amplification efficiency should have the following conditions:
1.The amplified product peaks earlier (the CT value is small).
2. High PCR amplification efficiency (close to 100% of theoretical value).

Trouble shooting

1. No CT value (signal) appears
a. The amount of template is insufficient or the template is severely degraded. Note that the RNA template is easily degraded. It can only be stored at -20°C for a short period of time. Long-term storage should be stored at -80°C.
b. The template contains serious inhibitors.
c. No primers or probes are added.
d. No RT-PCR Enzyme Mix is added.

2. The negative control also showed obvious amplification curve
a. Reagents or environment are contaminated by amplification products. Be careful not to open the PCR tube after the PCR reaction is over.
b. High-concentration control standards (especially plasmid DNA) have the same pollution ability as PCR amplification products, and the aerosol pollutants produced are also not negligible.
c. The pipette with filter element and template should be used, and be careful not to mix pipettes in PCR I area and PCR II area.
d. If it is determined that the reagents are contaminated by the amplification product, all reagents should be replaced, and all the original reagents should be discarded.

3. The experiment is not reproducible

a. Inaccurate sample addition. Adding ROX Reference Dye can correct the error caused by sample addition. If the instrument conditions permit, you should try to add ROX Reference Dye to use, do not omit it.

b. There are differences in the temperature conditions of the instrument on the samples, that is, the temperature uniformity is not good. Try to put the PCR tube in the middle to avoid edge effects.

c. The template concentration is low. The lower the initial concentration of the sample, the worse the repeatability. If conditions permit, try to reduce the dilution factor of the template.

4. Low amplification efficiency

a. If this product will not be used for a long time, please store it at -20℃.

b. There are PCR reaction inhibitors in the reaction system. Generally, it is introduced when the template is added. The template should be diluted appropriately first, and then added to the reaction system to reduce the influence of inhibitors.

5. Abnormal amplification curve

a. Improper setting of baseline, etc. Re-operate according to the instrument manual.

b. Too much template. When the amplification curve peaks within 10 cycles, the template should be diluted 100 to 1000 times before use.

c. When doing RNA template gradient, the PCR result has no gradient correlation. It may be that the original template concentration is too high and the dilution concentration is not enough; or the original template concentration is too low.

d. The amplification curve does not show S-shape, but is linear. It may be that the original template contains more PCR inhibitors, so the amount of template should be reduced, or the template should be diluted before use.