Power Green One-step RT-qPCR Kit

Cat. No. / SPEC.: V6001-A / 200 rxns; V6001-B / 5000 rxns

Description

Power Green One-step RT-qPCR Kit is a one-step reverse transcriptation-fluorescence quantitative PCR kit. This product contains reverse transcriptase, Taq DNA polymerase, dNTPs, SYBR Green I dye and other reagents required for reverse transcription and qPCR reaction. By adding only RNA (such as viral RNA) and primers, reverse transcription and dye qPCR can be performed in the same reaction tube. This product reduces the steps of experimental operation, not only improves the efficiency of detection, but also reduces the risk of contamination. Kits are available in convenient Master Mix form. Power Green 1-step Enzyme Mix contains heat-resistant reverse transcriptase and antibody modified hotstart DNA polymerase, which can maintain stable reverse transcriptase activity at 55°C to ensure high reverse transcriptase efficiency. hotstart Taq combined with optimized buffering system ensures high sensitivity and specificity of Power Green One-step RT-qPCR Kit. 2X Power Green 1-step Reaction Mix contains an optimized buffer system and SYBR Green I dye for SYBR/FAM channel detection.

Components

Component	V6001-A (200 rxns, 20 μL/rxn)	V6001-B (5000 rxns, 20 μL/rxn)
2X Power Green 1-step Reaction Mix [a]	1 mL × 2	25 mL × 2
Power Green 1-step Enzyme Mix [b]	200 μL	1 mL × 5

a. Contains dNTPs, SYBR Green I and raction buffer.

b. Contains Reverse Transcriptase, RNase inhibitor, and hot-start Taq DNA Polymerase.

Storage

Store at -20°C away from light, try to avoid repeated freezing and thawing, valid for 24 months.

Notes

① This product should avoid repeated freezing and thawing as far as possible, and can be stored at 4℃ away from light for short-term use. Before use, the Mix should be completely defrosted at room temperature, thoroughly mixed, and placed on ice for use.

② This product has high sensitivity, and attention should be paid to avoid non-specific amplification caused by aerosol contamination when preparing the reaction system.

③ Mixing (n+x) parts of the reaction liquid into n single tubes can reduce the sampling error. (n is the number of repetitions, x is the loss, generally 1/10 of n).

④ Gently mix the reaction solution to avoid bubbles, which can interfere with fluorescence detection. Instantaneous centrifugal removal of bubbles.

⑤ The specificity, dosage and annealing temperature of primers are important factors affecting the experimental results. It is necessary to design primers with good specificity, adjust the amount of primer appropriately with the experimental results (0.05-0.9μM) -- reduce the amount of primer when the specificity is poor, or increase the annealing temperature in an increment of 3℃, and increase the amount of primer when the amplification efficiency is low.

⑥ The amount of DNA template should be less than 1000 ng/ reaction, too high the amount of template will cause non-specific amplification. The dosage should be adjusted appropriately according to the template type and gene expression level.

⑦ The acquisition of the melting curve is not necessary and is recommended for the first primer. The specificity of the product can be seen by the melting curve. The reasons for the poor specificity of the product include: low specificity of primer; low annealing temperature setting, high primer/template concentration, etc. It is also suggested that the specificity of the product be detected by agarose gel electrophoresis.

⑧ This product does not contain reference dyes, please choose according to the instrument type and experimental requirements. The following table is for reference only:

Instruments	Final Conc. of ROX
ABI PRISM 7000/ PRISM 7700/ 7300/ 7900HT/ StepOne/ StepOnePlus/ GeneAmp 5700	500 nM，high ROX
ABI 7500/ 7500 Fast/ ViiA 7/ QuantStudio 6/7/12K Flex; Agilent Stratagene Mx3000P/ Mx3005P/ Mx4000	50 nM，low ROX
Bio-Rad CFX96/ CFX384/ iQ/ iQ5; MJ Research Opticon 2/ Chromo 4; Roche LightCycler 480/ 96; Corbett Rotor Gene G/ Q/ 3000/ 6000; Thermo PikoReal 96; Eppendorf MasterCycler ep realplex; Cepheid Smart Cycler	No ROX

Protocol

1. Preparation of reaction solution

Add the following reagents to the proper thermal cycler reaction tube or plate on ice:

Component	Volume	Final concentration
2X Power Green 1-step Reaction Mix	10 μL	1 ×
Power Green 1-step Enzyme Mix	1 μL	–
Forward Primer (10 μM)	0.4 μL	0.2 μM
Reverse Primer (10 μM)	0.4 μL	0.2 μM
Template RNA	variable	<1 μg
RNase-free ddH_2O	To 20 μL	–

The amount of each component in the reaction system can be adjusted according to the following principles:

• The optimal range for primers is 0.1~1.0 μM. In general, the primers with a final concentration of 0.2 μM work well.

• The length of the amplification product should be in the range of 80-200 bp.

2. Perform One-step RT-qPCR

Select the "SYBR" or "SYBR/FAM" channel according to the quantitative instrument, and refer to the following reaction procedures for detection:

Stage		Temperature	Time	Cycle
Reverse transcription		50~55°C [a]	10 min	1
Initial denaturation		95°C	1 min	1
Amplification	Denaturation	95°C	10 sec	40
	Annealing&Extension [b]	60°C	30 sec	
Melting curve analysis（optional）[c]		95°C	15 sec	1
		60°C	60 sec	
		95°C	15 sec	

a. For templates with complex secondary structures or high GC regions, increasing the reverse transcription temperature to 55ºC is conducive to improving amplification efficiency and sensitivity.

b. The extension time should be adjusted according to the minimum time limit of data collection required by the Real Time PCR instrument you use: at least 30 seconds with ABI 7700 and 7900HT; at least 31 seconds when using ABI 7000 and 7300; use ABI 7500 for at least 34 seconds.

c. Whether the Real Time PCR instrument actually used supports rapid amplification cycle or not, please conduct preliminary experiment to confirm the initial attempt.

3. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

Quality Control

Purity test: the absence of endodeoxyribonucleases, exodeoxyribonucl- eases and ribonucleases is confirmed by appropriate quality tests.

Functional test: the product has excellent specificity, sensitivity and repeatability after testing with different templates and primers from different sources.

Product Use Limitations

This product is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.