Product information

Product Name: One-Step qRT-PCR SYBR Green Kit

Catalog No: VN740RSK, VN741RSK

Packing Size: 500 x 25 µl rxns, 250 x 25 µl rxns

Shipping Condition: Ice pack / dry ice

Storage Condition: -20^{0}C

Product Description:

The One-Step qRT-PCR SYBR Green Kit is specifically designed for real-time quantitative analysis of RNA target genes. The kit components have been optimized for maximum sensitivity, specificity, and low background. The hot-start and inhibitor-resistant Taq polymerases included in the kit reduce non-specific PCR amplification and false negatives caused by PCR inhibitors, which are commonly observed with other commercial Taq polymerases.

While some PCR instruments require a passive reference dye (such as ROX), the One-Step qRT-PCR SYBR Green Kit does not contain ROX. However, you may add ROX to the reactions if your instrument requires it. Please refer to the table below for further details.

Final concentration of ROX dye in PCR	Recommended Cyclers
500 nM	ABI® PRISM 7000,7300,7700,7900HT, 7900Fast, StepOnePlus™, StepOne™
50 nM	ABI® PRISM 7500, 7500Fast -Stratagene® Mx3000, Mx3005P, Mx4000
No	BioRad® iCycler®, iQ ™5, MyiQ™ BioRad® CFX96 Roche LightCycler® 480 MJ Research Opticon™ And Opticon™ 2, MJ Research Chromo® 4 Corbett Rotor-gene® 6000, 3000 DNA Engine Option® 2 and Chromo 4™ Eppendorf® Realplex

List of Components:

- RT-PCR Pols: 2 x 250 µl (500 rxns) and 1 x 250 µl (250 rxns)
- 2X RT-PCR Mix: 5 x 1.25 ml (500 rxns) and 3 x 1.05 ml (250 rxns)

Protocol:

1. ***Thaw template RNA, primer solutions, and RT-PCR reagents, and place them on ice.***

   It is important to mix the solutions completely before use to avoid localized differences.

2. ***Prepare a master mix according to Table 1.***

   The master mix typically contains all the components required for RT-PCR except the template RNA. A negative control (without template RNA) should be included in every experiment.

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Table 1

PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
2X qRT-PCR SYBR Green Mix	12.5 µl	
Left Primer	Variable	0.2-0.4 µM
Right Primer	Variable	0.2-0.4 µM
SYBR Green	Variable	0.25-1X
ROX (not included)	Variable	0-500 nM
RT-PCR Pols	1.0 µl	
RNA template†	Variable	0.1 pg -1 µg
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-

†RNA amount depends mostly on genome size and target gene copy number.

3. ***Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.***
4. ***Add RNA template to the individual PCR tubes.***
5. ***Program the thermal cycler according to the program outlined in Table 2.***

   Table 2A and 2B describes a typical thermal cycler program. The program includes steps for both reverse transcription and PCR. Temperatures and cycling times can be further optimized for each new target and primer pair.

6. ***Start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 50-55°C and then place the PCR tubes in the thermal cycler.***

Table 2. Typical Cycling Parameters

Three steps

RT	50-55^{0}C	30 min
Initial denaturation	95^{0}C	5-10 min
Then followed by 35-45 cycles		
Denaturation	94^{0}C	20-30 sec
Annealing	50^{0}C to 68^{0}C	20-30 sec
Plate read	Follow instrument guideline	
Extension	70^{0}C	0.5-2 min
Melting curve	Follow instrument guideline	

Two steps

RT	50-55^{0}C	30 min
Initial denaturation	95^{0}C	5-10 min
Then followed by 35-45 cycles		
Denaturation	94^{0}C	20-30 sec
Annealing / Extension	60^{0}C to 65^{0}C	0.5-2 min
Plate read	Follow instrument guideline	
Melting curve	Follow instrument guideline	

Troubleshooting guide

No signal	Please check your PCR settings to ensure that you have not missed any necessary components. You may want to try a gradient annealing temperature to identify the optimal temperature for your target. Additionally, it appears that the annealing and extension times may be too short. Please refer to the protocol for the recommended times. If you are still experiencing issues, it is possible that the primers or RNA template are degraded or that the quantity of template is too low.
Low signal	If you are experiencing a low signal in your real-time PCR, there are several potential solutions. First, you may want to consider increasing the number of PCR cycles. Additionally, it is important to ensure that the reagents are stored properly to maintain their stability. It is possible that the primers are not optimal, in which case you may need to redesign them. Finally, the length of the PCR product may be contributing to the low signal; optimal PCR products are typically between 100-200 bp in length.
Unexpected variation	If you are experiencing unexpected variations in your real-time PCR results, there are a few steps you can take to address the issue. First, check your pipette to ensure that it is calibrated and accurate. Additionally, you should verify whether you need to add a ROX reference dye that is suitable for your specific PCR cycler model (please refer to the manufacturer's instructions or the provided reference table).

Note: This product is for R&D use only

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