Product information

Product Name: One-Step RT-PCR Kit

Catalog No: VN900RK, VN901RK

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

Shipping Condition: Ice pack / dry ice

Storage Condition: -20^{0}C

Product Description:

The One-Step RT-PCR Kit is designed for the amplification of RNA target genes from purified RNA. The kit components have been optimized for maximum sensitivity and specificity. A unique combination of enzymes and a specially developed reaction buffer ensures efficient and highly specific reverse transcription and PCR in a single tube.

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.

Table 1. PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-
2X RT-PCR Mix	12.5 µl	1X
Left Primer	Variable	0.2-0.5 µM
Right Primer	Variable	0.2-0.5 µM
RT-PCR Pols	1.0 µl	
RNA template	Variable	0.01 pg – 1 µg

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.***

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Table 2. Typical Cycling Parameters

2A. Three steps:

RT	50-55^{0}C	30 min
Then denaturation	95^{0}C	5-10 min
Then followed by 35-45 cycles		
Denaturation	94^{0}C	20 to 30 sec
Annealing	50^{0}C to 68^{0}C	20 to 30 sec
Extension	70^{0}C	1-2 min / kb
Final Extension	70^{0}C	10 min

2B. 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 to 30 sec
Annealing / Extension	60-65^{0}C	1-2 min / kb

Troubleshooting guide

No product or low yield	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. If you are experiencing a low yield in your RT-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.
Non-specific bands are observed	Set up the RT-PCR reaction on ice to avoid premature cDNA synthesis. Ensure that the thermal cycler is preheated to 50-55°C before adding the samples. The recommended temperature range for the RT reaction is 50-55°C. If necessary, increase the annealing temperature in increments of 2°C. Consider performing a touchdown PCR to optimize the reaction conditions
Product is smeared	If too much starting RNA was used, it is recommended to serially dilute the template RNA from stock solutions and repeat the RT-PCR using the new dilutions. If the enzyme concentration is too high, titrate the enzyme concentration from 0.5 µl to 1.0 µl per reaction. To avoid carry-over contamination, exchange all reagents and use pipette tips containing hydrophobic filters. Additionally, set up all reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.

Note: This product is for R&D use only

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