

PRODUCT INFORMATION

Cat. No.
R-4000 1 mL

Description: *Prime* RT-PCR Premix (2X) contains *HS Prime Taq* DNA Polymerase (hot-start *Taq* DNA Pol.), *Prime* MMLV RTase, reaction buffer, dNTPs mixture, RNase Inhibitor, protein stabilizer and enhancers for cDNA synthesis and PCR. Also, *Prime* RT-PCR Premix (2X) optimizes the convenience to use by adding sediment for electrophoresis and solution of loading dye.

Best Stability and Reproducibility: As each tube of *Prime* RT-PCR Premix (2X) contains a stabilizer, which can maintain the stability of the *Prime* MMLV RTase and *Prime Taq* DNA Polymerase up to a year at -20°C.

High Yield (Use the Hot start *Taq* DNA Pol.):

Prime RT-PCR Premix (2X) contains a monoclonal *Prime Taq* antibody and enhancers for cDNA synthesis and DNA amplification. In most applications an increase in yield observed as compared to the standard reactions.

USAGE INFORMATION

Optimal reaction conditions, such as amount of RNA and primers, may vary and must be individually determined.

General Reaction Protocol:

1. Mix the template RNA and the primer in RNase-free tube and add 10 µL of *Prime* RT-PCR Premix (2X).

Note: if you use the Cat. No. R-5000 (8-strip, 10 µL X 96 tubes), transfer the mixture (template RNA + specific primer = 10 µL) to *Prime* RT-PCR Premix (2X, 10 µL) tube.

Note: Concentration of template RNA and primer (20 µL reaction volume)

Template RNA	Total RNA	below 1 µg
	Poly(A) ⁺ mRNA	50 ng~0.1 µg
Specific primer	forward primer	10~20 pmole
	reverse primer	10~20 pmole
RNase-free (DEPC-treated) water		Up to 10 µL
<i>Prime</i> RT-PCR Premix (2X)		10 µL
Total reaction volume		20 µL

2. Mix by pipetting gently up and down (total reaction volume 20 µL).

3. (Optional) Add mineral oil.

Note: This step is unnecessary when using a thermal cycler with top heating method.

4. First strand cDNA synthesis and DNA amplification.

Step	Temp.	Time	
cDNA synthesis	42°C	30 min.	1 cycle
Inactivation of RTase for activation of <i>HS Prime Taq</i> Pol.	94°C	10 min.	
DNA amplification	Annealing temperature and time need to be optimized for each primer and template combination		
Final extension	72°C	5 min.	

5. Separate the PCR products by agarose gel electrophoresis without adding a loading dye.

Note: A DNA fragment which is amplified by *Prime* RT-PCR Premix has A-overhang, and it enables you to do cloning by using T-vector.

Product Use Limitations

This product is sold for research purposes only. This is not to be used for human diagnostic or drug purposes. All claims must be brought within expired date.

User Notes

Store at -20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.