Ultra Thermo-stable M-MLV Reverse Transcriptase (RNase H-)

User's Instruction

Description

Ultra Thermo-stable M-MLV Reverse Transcriptase (RNase H-) is a mutant type of M-MLV Reverse Transcriptase, obtained by eliminating the active center of RNase H through multiple point mutations. The alteration decreases activity of RNase H and reduces RNA degradation in reverse transcription, which increases the yield of first-strand cDNA to get full length cDNA more easily.

In addition, thermal stability of the reverse transcriptase is improved and the enzyme is active up to 65°C. Compared with RT-PCR at low temperature, RT-PCR at high temperature can significantly open the secondary structure of RNA, promote the amplification performance of complex RNA template, increase the length and yield of cDNA, and improve the sensitivity of subsequent detection.

Kit Contents

	10KU
1. Ultra Thermo-stable M-MLV Reverse Transcriptase (RNase H-)	50 μl
2. 5×RT Buffer	1 ml

Unit Definition

One active unit is defined as the amount of enzyme needed to catalyze the incorporation 1 nmol dTTP into acid insoluble sediment in 10 min under 37°C with poly(rA) as template and oligo(dT) as primer.

Protocol

1. Set up the reaction as the following table (take 20 μl per well as an example):

Component	Volume
Ultra Thermo-stable M-MLV Reverse Transcriptase (RNase H-)	0.5-1 μl
5×RT Buffer	4 μl

dNTP Mixture (10 mM each)	1 µl
Total RNA or Poly(A) RNA	0.1-2 µg
*20×Oligo dT(25) & Random Primer	1 µl
RNase Inhibitor (40 U/µl)	0.5 µl
RNase Free H_2O	Up to 20 µl

- *The concentration of oligo dT(25) should be 20-50 µM. Random primers should be used at 125 µM while gene specific primers should be 5 µM.

2. Thermocycling Conditions

   a) 30°C for 5 min

   b) *37-65°C for 15-60 min

   c) 85°C for 10 min

   d) 4°C

   - *Generally, when the reaction temperature is set at 50°C, comprehensive cDNA production can be obtained regardless of high GC or long template. When the reaction temperature is set at 65°C, there will be a significant increase in the cDNA production for the templates with >75% GC content.

3. The cDNA product can be directly used for the downstream PCR reaction or stored at - 20°C.

Storage

Minimum shelf life is 3 years at -20°C.