Thermostable Reverse Transcriptase [M-MLV, RNase H-]

User's Instruction

Product Concentration: 200 U/μl

Storage: -20℃, 2 years

Description

Thermostable Reverse Transcriptase is a recombinant M-MLV reverse transcriptase with reduced RNase H activity and increased thermostability. The enzyme is active up to 55℃. It provides higher specificity, higher yield and more full-length cDNA products.

- Increased thermostability for more full-length cDNA products.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- cDNA up to 15 kb.

Unit Definition

One activity unit (U) refers to the amount of M-MLV reverse transcriptase when catalyzes the incorporation of 1 nmol of dTTP into materials in 10 min at 37℃ using oligo (dT) primed poly (A) as a template.

5 X RT Buffer

250 mM KCl; 15 mM $MgCl_2$; 10 mM DTT; 100 mM Tris-HCl pH 8.4.

First Strand cDNA synthesis (20 μl reaction volume)

1. Add components according to the below table:

Components	Volume
Total RNA/mRNA	50 ng-5 μg/5-500 ng
Oligo$(dT)_{18}$ (0.5 μg /μl)	1 μl
Or random Primer (0.1 μg/μl)	1 μl
Or GSP (Gene Specific Primer)	2 pmol
dNTP Mix, 10 mM each	1 μl
5 X RT Buffer	4 μl
Ribonuclease Inhibitor (40 units/μl)	0.5 μl
Thermostable H^- RTase	1 μl
RNase free H_2O to final volume	20 μl

Optional (if RNA template is GC-rich or is known to contain secondary structures). Suggest to mix RNA /Primer/RNase free H_2O gently and briefly centrifuge, incubate at 65℃ for 5 min, chill on ice and briefly centrifuge, then place the tube on ice. Add other components and continue.

2. Mix well gently

If Oligo(dT)$_{18}$ or gene specific primer (GSP) are used, incubate at 50℃ for 30-50 min.

If Random Primer is used, incubate 10 min at 25℃ followed by 30-50 min at 50℃.

3. Terminate the reaction by heating at 70℃ for 15 min.

   The reverse transcription reaction product can be directly used in PCR or stored at -20℃.

RT-PCR

Use 2-4 μl of the reaction mix to perform PCR in 50 μl volume.

PCR mixture set up (for 50 μl reaction volume)

Components	Volume	Final Concentration
cDNA Template	2-4 μl	as required
Forward Primer (10 μM)	1 μl	0.2 μM each
Reverse Primer (10 μM)	1 μl	0.2 μM each
10X Taq Buffer (contains Mg^{2+})	5 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
Taq DNA Polymerase	0.5 μl	2.5 units
ddH_2O to final volume	50 μl	Not applicable

PCR Condition

94℃	2-5 min	
94℃	30 sec	30-40 cycles
50-60℃	30 sec	
72℃	1-2 kb/min	
72℃	5-10 min	