TOOLS M-MLV RTase

Cat. no. TTF-MLV01

Product Size:

Contents	Product size
TOOLS M-MLV RTase	20,000 U (200 U/μL , 100 μL /vial)
5X RT Buffer	1 mL

Storage: -20℃ for 1 year

Description

TOOLS M-MLV RTase is an RNA-dependent thermostable DNA polymerase that synthesizes the first strand of cDNA from a single-stranded RNA template with hybridized primer. This polymerase is purified from RNase H mutant E. coli and exhibits lower effect of the secondary structure of RNA during cDNA synthesis.

5x RT Buffer

250 mM Tris-HCl (pH 8.3), 15 mM MgCl2, 375 mM KCl, 50 mM DTT

Storage Buffer

20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.25 mM EDTA, 0.01% NP-40 (v/v), 2.5 mM DTT, 50% glycerol (v/v).

Applications and Features

- Low RNase H activity. High yield of long cDNA synthesis.
- Thermal stable: (50-60°C).
- Wider temperature range: (37-60°C).
- Strong amplification activity: The RNase H mutant enzyme enhances the binding capacity of the enzyme and RNA resulting increased amplification speed and yields high quality cDNA
- Suitable for cDNA library construction.

Unit Definition

One unit is defined as the amount of the enzyme incorporates 1 nmol of dTTP into acid-insoluble product in 10 minutes at 37°C.

Protocol

Synthesis of first-strand cDNA 20 μL reaction system can be used for reverse transcription of 1-5 μg total RNA or 50-500 ng mRNA.

1. Add the following components to a nuclease-free micro-centrifuge tube.

Component	Volume	Final concentration
Choose one of the following primers		
Oligo (dT) $^{12-18}$ (50 μM)	1 μL	Oligo (dT) $^{12-18}$ 2.5 μM
Random primer mix (50 μM)		Random primer mix 2.5 μM
Gene-Specific primer (2 μM)		Gene-Specific primer 0.1 μM
Total RNA or mRNA	X μL	1 ng -5 μg of total RNA
dNTP mixture (10 mM)	1 μL	
RNase-free ddH_2O	Y μL	
Total Volume	10 uL	

2. Heat at 65 °C for 5 min, and place the tube immediately on ice for 2 min. Short centrifuge for 2-3 seconds then add:

Component	Volume	Final concentration
5X Reverse Transcriptase reaction buffer	4 μL	1X
TOOLS M-MLV Rtase(200U/μL)	1 μL	
RNase Inhibitor	X μL	8 U/rxn
RNase-free ddH_2O	Y μL	
Total Volume	10 uL	

3. Incubate at 42°C for 1 hour. If random primer mix is used, incubate the reaction at 25 °C for 10 min
4. Heat the sample to 65°C for 20 min to inactivate TOOLS M-MLV RTase.
5. The cDNA products should be store at -20 °C
6. Take 2-5 μL for PCR of qPCR amplification.

The product is for research only, not for diagnostic and clinical use.