Thermo-resistant H Minus M-MuLV Reverse Transcriptase

Description: This a genetically modified RNA-dependent DNA polymerase requiring a DNA primer and an RNA template to synthesize a complementary DNA strand. Thermo-resistant H Minus M-MuLV Reverse Transcriptase has no RNase H activity. Therefore, degradation of RNA does not occur during first strand cDNA synthesis, resulting in higher yields of full-length cDNA from long templates compared to other reverse transcriptases. Thermo-resistant H Minus M-MuLV Reverse Transcriptase maintains activity over a wide temperature range (42-52°C) which makes it an ideal tool for reverse transcription of RNAs having a high degree of secondary structure.

Contents:

Component	50 Rxn
Thermo-resistant RT	100 µL
5X RT Buffer	500 µL

Kit storage:

This kit should be stored at -20°C. Under these conditions reagents are stable for one year from the date of production.

Protocol (first strand cDNA synthesis):

1- Mix the template RNA (total RNA or Poly(A)mRNA) and the primer in RNase-free tube as below table. Optimal reaction conditions, such as amount of RNA and primers, may vary and must be individually determined. Random hexamer or oligo (dT)16 or specific primers could be used as primer.

Concentration of template RNA and primer		
Template RNA	Total RNA or Poly(A)+ mRNA	10 ng~5 µg 5 ng~0.5 µg
Primer	Oligo (dT)16 or Random hexamer	1-2 µL 1 µL
DEPC-treated water		Up to 12 µL (11 µL*)

* If you use RNase inhibitor

2- Incubate the mixture at 65 °C for 5 min and chill on crash ice and add the reagent as follow:

Concentration of template RNA and primer	
5X RT Buffer	4
RNase Inhibitor 20 U/ul (optional)	1
10 mM dNTP Mix	2
Thermo-Resistant RT	2

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

4- Incubate 10 min at 25 °C (omit this step for Oligo dt).

5. Incubate 60 min at 47 °C.

6. Stop the reaction by heating at 70 °C for 10 minutes. Chill on ice.