



## M-MLV reverse transcriptase H<sup>-</sup>

Cat No	Pack size	conc
RT0100	10000 U	200U/ul
RT0500	50000 U	200U/ul

### Description:

MMLV Reverse Transcriptase, encoded by Moloney Murine Leukemia Virus (M-MLV RT) is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template to which a primer has been hybridized. MMLV RT will also extend primers hybridized to single-stranded DNA. Second strand cDNA synthesis can be achieved from some RNA templates without an additional DNA polymerase M-MLV RT (H<sup>-</sup>) can synthesized 9.5kb products, the largest RNA component in the reaction. However, M-MLV RT synthesized more Full-length cDNA regardless of size

**Storage conditions:** -20°C

### Unit definition:

One unit of activity is the amount of enzyme required to incorporate 1 nmole of dTTP into an acid-insoluble form in 10 minutes at 37°C using polyA-oligo (dT) as template and primer.

### Supplied 5xRT buffer :

250 mM TrisHCl, pH 8.3  
375 mM KCl  
15 mM MgCl<sub>2</sub>  
50 mM DTT

### Protocol

1. Mix in the tube: 1-5  $\mu$ g of the total RNA (or 50-500 ng of polyA RNA) 10 pmole of strand-specific primer (or 250 to 500 ng of oligo -dT or random primer for each  $\mu$ g of RNA) add water up to 8  $\mu$ l
  2. Incubate the mixture 10 min at 70°C, stand 10-15 min at room temperature or place on ice
  3. Add into the mixture:
    - 4  $\mu$ l of 5xRT buffer
    - 1  $\mu$ l of dNTP mix 10mM
    - RNAsin – 20-40 units (optional)
    - 1ul M-MLV RTase – 200 units
    - H<sub>2</sub>O – up to 20  $\mu$ l
  4. Incubate the mixture at 37-50°C during 30-120 min. The time of reaction depends on the length of cDNA, 30 min is for cDNA in range of 500 bp, 120 min is for cDNA more than 1.5 kb. The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 50°C) for the highly structured RNA.
  5. Heat the mixture 10 min at 65-70°C to inactivate the RTase.
  6. Use the mixture for PCR or for other application.
- For your PCR-Reaction you need 1-10  $\mu$ l of your RT-PCR product.

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