



RB MMLV Reverse Transcriptase (RNase H-)

Source:

Recombination of E.coli containing Moloney murine leukemia virus mutated reverse transcriptase gene from clone of Moloney murine.

Concentration: 200U/ μ l

Component: M-MLV (400U/ μ l) , 5 \times Buffer (with DTT)

Features:

lack RNase H activity: Weak RNaseH activity High cDNA yield, can get more full length cDNA.

thermal stable: the optimum reaction temperature is 50C, the highest is 60C. Can overcome the template RNA secondary structure, and finish the reverse transcriptase experiment smoothly.

wide temperature range: can reverse transcript from 37-60C, with more than 80% of the highest activity

at 42C-55C customer can choose the reaction temperature freely.

Strong amplification activity: Gene mutation enhanced the binding capacity of the enzyme and RNA. Increased the amplification speed, can obtain the quality cDNA, suitable for cDNA library construction.

Storage: -20C

Unit Definition:

One unit of MMLV RT catalyzes the incorporation of 1 nmol of dTTP into acidinsoluble material in 10 minutes at 37C using oligo(dT)₁₂₋₁₈-primed poly(A)_n as a template.

Applications:

The first-strand cDNA synthesis; RT-PCR.

Storage Buffer:

20 mM Tris-HCl (pH7.5), 200 mM NaCl, 0.25 mM EDTA, 0.01% NP-40(v/v), 2.5 mM DTT, 50% glycerol (v/v).

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5X Reaction Buffer:

[5×RT Buffer] 250mM Tris-HCl (pH 8.3), 15mM MgCl₂, 375 mM KCl, 50mM DTT.

Preparation of RNA for RT-PCR

1. Add to an RNase-free PCR tube:
2. 8-10 μ L RNA dissolved in double distil water (500 ng-5 μ g)
3. 1 μ L of DNase I, Amplification Grade, 2 unit/mL
4. 1 ul DNase I, buffer
5. Mix gently with hand not vortexing, and incubate for 15 minutes at 37 C°

Recommended Reaction Conditions:

The first-strand cDNA synthesis

- | | |
|--|-----------------------|
| 1. Oligo dT12-18 (1 μ g/ μ l) or random primer (50-250ng) | 1 μ l |
| 2. Total RNA (10ng-5 μ g) or mRNA(10-500ng) | 4-5 μ l |
| 3. dNTP (10mM each) | 1 μ l |
| 4. DEPC ddH ₂ O | up to (10-12) μ l |
| 5. Gently mix and SHORT SPINE then incubate 10 Min at 65C then chilled on ice for 8-10min. | |
| 6. 5×RT Buffer | 2-4 μ l |
| 7. Gently mix and do a short spine | |
| 8. Add 1 μ l M-MLV RT (200U/ μ l), Incubate at 50C for 50min. | |

Inactivate at 72C for 15min then get the CDNA

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