| Product Name | Cat. No. | Pack Size |
|---|---|---|
| **Super Rev. Transcriptase MuLV KIT (Super RT 50 Rxns KIT)** | **# BB-E0043** | **10,000 U (200 U/µl)** |

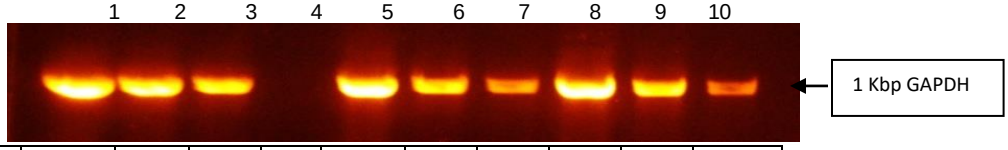
**Description:** Murine Leukemia Virus Reverse Transcriptase (MuLV RT) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (>5Kbp). The enzyme is isolated from *E.coli* expressing modified *pol* gene of MuLV on a plasmid. ***This modified version of RT is thermotolerant (working temp 42 – 52 ºC using ng level of total RNA).*** The RNase H activity of MuLV RT is weaker than the commonly used AMV- Reverse Transcriptase.

**Reagents supplied with the KIT**

1. Super RT (200 U/µl), 100 µl
2. 5 x RT Buffer (250 mM Tris-HCl, pH 8.4; 375 mM KCl; 15 mM $MgCl_2$), 400 µl
3. dNTP mix (10mM), 50 µl
4. Oligo (dT) mix (500 µg/ml), 50 µl
5. Random Hexamer (50 ng/ul), 50 ul
6. 0.1 M DTT, 100 µl
7. Murine RNase Inhibitor (1 U/µl), 50 µl
8. DEPC $H_2O$, 600 µl

**Storage Buffer:** 20mM Tris-HCl (pH 7.5), 100mM NaCl, 0.1mM EDTA, 1mM DTT, 50% glycerol (v/v).

**Storage Instruction**: **- 20ºC**




| Lane No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Total RNA (ng) | 50 | 10 | 5 | 50 | 50 | 10 | 5 | 50 | 10 | 5 |
| RT (U) | 200 | 200 | 200 | 0 | 200 | 200 | 200 | 200 | 200 | 200 |
| Temp (º C) | 42 | 42 | 42 | 42 | 50 | 50 | 50 | 52 | 52 | 52 |

Reverse Transcription done with the 200 U of Super-RT (MuLV) using above mentioned amount of total RNA in a 20 µl reaction volume. 2 µl of RT product used for the PCR of 1 Kbp amplicon of GAPDH.

## First-Strand cDNA Synthesis Using Super RT Enzyme

- Normally, a 20 µl Super RT reaction volume uses 5ng/10ng of Total RNA or (1ng to 2 ng of mRNA) for reaction at temperature 42$^{O}$C. However, depending on the nature of reaction the input template total RNA or mRNA may be enhanced and reaction temperature may also be chosen at a workable temperature between 42ºC to 52ºC.

- Add the following components to a nuclease-free microcentrifuge tube

| | |
|---|---|
| **Use** Oligo $(dT)_{12-18}$ (500 µg/ml)<br>**or,** Blend of random Hexamer (50 ng / µl ) & Oligo$(dT)_{12-18}$ (3:1) v/v<br>**or,** 2 pmole gene-specific primer (GSP) | 1 µl |
| 5 ng to 10 ng total RNA OR 1 ng to 2 ng of mRNA OR more total RNA / mRNA | X µl |
| 1 µl dNTP Mix (10mM each) | 1 µl |
| Sterile, distilled water | Up to 12 µl |

- Heat mixture to 65ºC for 5 min. & quick chill on ice. Collect the contents of the tube by brief centrifugation and add (preferably make a master mix and add accordingly):

| | |
|---|---|
| 5 X First-Strand Buffer | 4 µl |
| 0.1 M DTT | 2 µl |
| RNase Inhibitor | 1 µl |

- Mix contents of the tube gently. Keep it at room temp for 5 min.
- Add 1 µl of Super RT (200U/µl) and mix by pipetting gently (softly) up and down
- Incubate at **42ºC - 52°C** for 50 min (depending on your template)
- Inactivate the reaction by heating at 70ºC for 15 min.

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