

Product Name	Cat. No.	Pack Size
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Super Reverse Transcriptase MuLV	# BB-E0042	20,000 U (100 µl)
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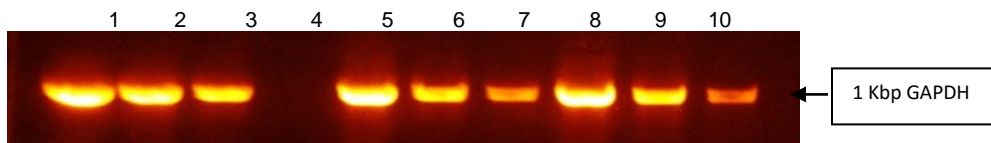
Description: Murine Leukemia Virus Reverse Transcriptase (MuLV RT) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (>5Kbp). The enzyme is isolated from *E.coli* expressing modified *pol* gene of MuLV on a plasmid. **This modified version of RT is thermotolerant (working temp 42 – 52 °C 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

- Super RT (200 U/µl) , 100 µl
- 5 X RT Buffer (250 mM Tris-HCl, pH 8.4; 375 mM KCl; 15 mM MgCl₂), 400 µl

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

Storage Instruction: - 20°C



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 (°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.



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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 2ng of mRNA) for reaction at temperature 42°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 42°C to 52°C

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

Use Oligo (dT) ₁₂₋₁₈ (500 µg/ml) or, Blend of random Hexamer (50 ng / µl) & Oligo(dT) ₁₂₋₁₈ (3:1) v/v 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 65°C 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 **42°C - 52°C** for 50 min (depending on your template)
- Inactivate the reaction by heating at 70°C for 15 min.

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