

**M-MuLV Reverse Transcriptase, RNase H Minus
(20000 Units)**

Cat No.110053

DATA-SHEET

M-MuLV Reverse transcriptase is purified from E.coli strain harbouring a plasmid that directs the synthesis of modified form of Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase. M-MuLV reverse transcriptase is an RNA/ DNA directed DNA Polymerase. The enzyme can synthesize complementary DNA strand initiating from a primer using either RNA (cDNA Synthesis) or single stranded DNA as template. This enzyme had been genetically altered to remove associated RNase H activity. Removal of RNase H activity resulted in an increase of full-length cDNA products. Molecular weight of Reverse is 69 kDa.

UNIT DEFINITION :

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

CONCENTRATION SUPPLIED : Enzyme is supplied at a concentration of 200U/μl along with one vial of 5X buffer, one vial of additional MgCl₂ & one vial of DNA's free water.

Storage Buffer : 50mM Tris-HCl (pH 8.3), 1mM EDTA, 100mM NaCl, 0.1mM DTT, 0.1% Triton X-100 and 50% Glycerol.

CONTENTS OF THE PACK :

20000 units of M-MuLV reverse transcriptase	50 μl	- 2 Vial
5 X Reaction buffer	1.5 ml	- 1 Vial
25 mM MgCl ₂	1.5 ml	- 1 Vial
Mol. Grade water	1.5 ml	- 1 Vial

5X Reaction Buffer complete including Magnesium (3mM final 1x Conc.)

250mM Tris-HCL (pH 8.3), 500mM KCl, 50mM DTT and 15mM MgCl₂

Mol grade water: Water is supplied in 1.5ml x 1 quantity, which is RNase and DNase free. Advisable to aliquot the Mol. Grade water.

MgCl₂ Solution : 1.5 ml of 25mM MgCl₂ Solution (In case higher Conc. of Mg is to be used)

Storage Conditions : Recommended storage temperature is -20 °C.

AVIALABLE PACK SIZE :

Cat. No.	PRODUCT	Pack Size
110051	M-MuLV Reverse Transcriptase	5000 U
110052	M-MuLV Reverse Transcriptase	10000 U
110053	M-MuLV Reverse Transcriptase	20000 U (10000 U x 2)
110054	M-MuLV Reverse Transcriptase	50000 U (25000 U x 2)
110055	M-MuLV Reverse Transcriptase	100000 U (25000 U x 4)



Manufactured and Marketed By :

GENOME DIAGNOSTICS PVT. LTD.,
(An ISO 13485:2003 & ISO 9001:2000 Certified Company)

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Suggested Protocol

cDNA synthesis with M-MuLV reverse transcriptase

- Mix in the tube :**
 - 1-5µg of total RNA (or 50 – 500ng of polyA RNA)
 - 10pmole of strand-specific primer (or 250 – 500ng of oligo-dT for each µg of RNA)
 - Add water to make the final volume upto 8µl
- Incubate the mixture** for 10 min at 65°C, then 10 – 15min at room temperature (for the specific primer or place in ice in case of oligo-dT or random primer)
- Add into the Mixture :**
 - 4µl of 5X RT buffer complete (250mM Tris-HCl, pH 8.3; 500mM KCl, 15mM MgCl₂)
 - 1µl of dNTP mix (5mM of each dNTP)
 - Rnasin: 20-40 units (optional)
 - M-MuLV Reverse Transcriptase – 200 units
 - Make the final Volume to 20µl with water supplied with this product.
- Incubate the mixture** at 37°C - 42°C for 30 to 120min. The time of reaction depends on the length of cDNA; 30min is sufficient for cDNA in the range of 500bp in length, 120min for 1.5 kb respectively. The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 50°C) for the highly structured RNA. The optimal temperature and reaction time should be adjusted for each particular RNA. We recommend to use buffer with pH 8.8 if the reaction is performed at elevated temperature.
- Heat the mixture** for 10min at 70°C to inactivate the M-MuLV Reverse Transcriptase.
- Use the mixture** for PCR and for other applications.

Note : M-MuLV Reverse Transcriptase and RT 5X buffer are free form RNase activity.

NOTE : For research use only. Not for use in Diagnostic or therapeutic procedures.
Genome Diagnostics Pvt. Ltd., shall not in any event be liable for incidental or special damage of any kind resulting from any use except on applications.



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