

> M-MLV Reverse Transcriptase

M-MLV Reverse Transcriptase is a recombinant DNA polymerase that synthesizes a complementary DNA strand from single-stranded RNA, DNA, or an RNA:DNA hybrid. Compared to AMV RT, Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) lacks DNA endonuclease activity and has a lower RNase H activity.

cat. no.	amount	note
STS-MRT 10	10000 units	5X Reverse Buffer
STS-MRT 50	50000 units	5X Reverse Buffer
STS-MRT 200	200000 units	5X Reverse Buffer

FOR RESEARCH USE ONLY

UNIT DEFINITION

One unit is defined as the amount of enzyme required to incorporate 10 nanomoles of dNTPs into acid-insoluble material in 30 min at 74°C.

STORAGE BUFFER

20mM Tris-HCl pH 8.0, 200mM NaCl, 1mM DTT, 0.5mM PMSF, 0.5% TRITON X-100, 50%(v/v) glycerol

SHIPPING

Shipped in green ice.

STORAGE

Store at -20C°

SHELF LIFE

12 months

FORM

liquid

CONCENTRATION

200U/ul

component	STS-MRT10	STS-MRT50	STS-MRT200
M-MLV Reverse T.	10000 units / 50ul	50000 units / 250ul	200000 units / 1ml
5X Reverse Buffer	1ml 5X Buffer	1ml 5X Buffer	4ml 5X Buffer
DDT	500ul / 100mM	500ul / 100mM	500ul / 100mM

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First Strand cDNA synthesis

Assay Set-Up:

Before starting, vortex all components thoroughly to ensure homogeneity.

Prepare a premix for the number of assays you need according to the following protocol:

First - Strand cDNA synthesis

Final volume: 20ul

- In a nuclease-free microcentrifuge tube add:
 - dNTPs mix (10mM each) 1ul
 - 1ng to 4ug total RNA
 - or
 - 1-500ng mRNA xul
 - 50-100ng random primers
 - or
 - Oligo(dT)12-18 (500ug/ml) 1ul
 - ddH₂O up to 12ul
- Heat mixture @ 65°C for 2min. and quick cool the tube on ice. Add:
 - 5X Reverse Buffer 4ul
 - 100mM DTT 2ul
 - ddH₂O 1ul
 - or
 - Ribonuclease Inhibitor (optional)
- Mix gently and incubate
 - @ 42°C for 2 min. if you are using oligo(dT)12-18
 - or
 - @ 25°C for 2 min. if you are using random primers
- Add 1ul (200U) of GeneSpin M-MLV Reverse Transcriptase (if you are using random primer incubate @25°C for 10 min.)
- Incubate @ 42°C for 60 - 120 min.
- Inactivate the reaction @ 70°C for 10 min.