



## M-MLV Reverse Transcriptase RNase H-

Cat. No.	Pack Size	Conc.
06-21-00000S	2000 U SAMPLE	200 U/μl
06-21-010000	10000 U	200 U/μl
06-21-050000	50000 U	200 U/μl
06-21-200000	200000 U	200 U/μl

For *in vitro* use only

### Description:

The M-MLV Reverse Transcriptase RNase H- is a genetically modified M-MLV Reverse Transcriptase which exhibits RNA or DNA dependent DNA polymerase, but lacks ribonuclease H activity. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA or DNA templates. Removal of the RNase H activity results in an increase of full-length cDNA products.

### Application:

- cDNA synthesis
- RNA analysis by primer extension
- DNA labeling

### Reagents Provided:

- **M-MLV Reverse Transcriptase RNase H-**
- **5 x RT Reaction buffer 1** (with MgCl<sub>2</sub> and DTT)  
*0.25 M Tris-HCl, 0.5 M KCl, 30 mM MgCl<sub>2</sub>, 25 mM DTT*
- **5 x RT Reaction buffer 2** (Mg<sup>2+</sup> and DTT free)  
*0.25 M Tris-HCl, 0.5 M KCl*
- **25 mM MgCl<sub>2</sub>**
- **20 mM MnCl<sub>2</sub>**
- **100 mM DTT**

### Concentration:

200 U/μl

### Unit definition:

One unit is defined as the amount of enzyme required to catalyze the incorporation of 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C.

### Storage and Dilution buffer:

50% glycerol (v/v), 50 mM Tris-HCl pH 8.0 at 25°C, 100 mM NaCl, 5 mM DTT, 1 mM EDTA, 0.1% NP-40.

### Quality control:

Free of endo- and exodeoxyribonucleases, phosphatases and ribonuclease. Activity and stability tested in first strand cDNA synthesis.

### Shipping and Storage conditions:

Routine storage: -20°C

Shipping at room temperature has no detrimental effects on the quality of this reagent.

### Recommended cDNA synthesis reaction mix: for 5 x RT Reaction buffer 1 (with MgCl<sub>2</sub> and DTT)

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/μl)	1 μl	4 U/μl
5 x RT Reaction buffer 1	10 μl	1 x
20 mM dNTP mix	0.5-1.25 μl	200-500 μM
100 mM DTT	0-2.5 μl	5-10 mM
RNase inhibitor *		optional
p(dT) <sub>25</sub> / random primer or gene-specific primer per μg of RNA		250-500 ng / 50-100 ng
RNA	2-5 μg	
H <sub>2</sub> O	Up to 50 μl	

### Recommended cDNA synthesis reaction mixes: for 5 x RT Reaction buffer 2 (Mg<sup>2+</sup> and DTT free)

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/μl)	1 μl	4 U/μl
5 x RT Reaction buffer 2	10 μl	1 x
20 mM MnCl <sub>2</sub>	5 μl	2 mM
100 mM DTT	2.5-5 μl	5-10 mM
20 mM dNTP mix	0.5-1.25 μl	200-500 μM
RNase inhibitor *		optional
p(dT) <sub>25</sub> / random primer or gene-specific primer per μg of RNA		250-500 ng / 50-100 ng
RNA	2-5 μg	
H <sub>2</sub> O	Up to 50 μl	

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/μl)	1 μl	4 U/μl
5 x RT Reaction buffer 2	10 μl	1 x
25 mM MgCl <sub>2</sub>	6-12 μl	3-6 mM
100 mM DTT	2.5-5 μl	5-10 mM
20 mM dNTP mix	0.5-1.25 μl	200-500 μM
RNase inhibitor *		optional
p(dT) <sub>25</sub> / random primer or gene-specific primer per μg of RNA		250-500 ng / 50-100 ng
RNA	2-5 μg	
H <sub>2</sub> O	Up to 50 μl	

\* Although M-MLV Reverse Transcriptase RNase H- is free of contaminating RNases, the use of RNase inhibitor is strongly recommended.



### Safety warnings and precautions:

This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water

*Some applications this product is used in may require a license which is not provided by the purchase of this product. Users should obtain the license if required.*

### Related products:

Product name	Pack size	Cat. No.
<b>dNTP MIX</b> (20 mM of each)	20 µmol	02-31-00020
<b>dNTP MIX</b> (20 mM of each)	100 µmol	02-31-00100
<b>dNTP SET</b> (100 mM)	4 x 25 µmol	02-21-00100
<b>dNTP SET</b> (100 mM)	4 x 100 µmol	02-21-00400

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## Protocol

Reaction volume 50 µl

1. In a sterile microcentrifuge tube, add RNA and primer(s) in a total volume of 15 µl water
2. Heat the tube at 70<sup>0</sup>C for 5-10 minutes, then 10-15 minutes at room temperature (for specific primer) or place in ice in case of p(dT)<sub>25</sub> or random primer
3. Spin for a few seconds
4. Add water
5. Add dNTPs
6. Add 5 x RT buffer 1 or  
5 x RT Reaction buffer 2 and DTT and MnCl<sub>2</sub> or  
5 x RT Reaction buffer 2 and DTT and MgCl<sub>2</sub>
7. Add RNase inhibitor (optional)
8. Add M-MLV Reverse Transcriptase RNase H-
9. Mix gently and incubate at 37<sup>0</sup>C for 30-90 minute