Solis BioDyne
Data Sheet

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-**
- **5x RT Reaction buffer 1** (with $MgCl_2$ and DTT)
  0.25 M Tris-HCl, 0.5 M KCl, 30 mM $MgCl_2$, 25 mM DTT
- **5x RT Reaction buffer 2** (Mg^{2+} and DTT free)
  0.25 M Tris-HCl, 0.5 M KCl
- **25 mM $MgCl_2$**
- **20 mM $MnCl_2$**
- **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 dNTP into an acid-insoluble form in 10 minutes at 37ºC.

Storage and Dilution buffer:

50% glycerol (v/v), 50 mM Tris-HCl pH 8.0 at 25ºC, 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: -20ºC
Shipping at room temperature has no detrimental effects on the quality of this reagent.

Recommended cDNA synthesis reaction mix:

for 5x RT Reaction buffer 1 (with $MgCl_2$ and DTT)

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/µl)	1 µl	4 U/µl
5x 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
Oligo(dT) / random primer or gene-specific primer per µg of RNA		250-500 ng / 50-100 ng
RNA	2-5 µg	
H_2O	Up to 50 µl	

Recommended cDNA synthesis reaction mixes:

for 5 x RT Reaction buffer 2 (Mg^{2+} and DTT free)

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/µl)	1 µl	4 U/µl
5x RT Reaction buffer 2	10 µl	1 x
20 mM $MnCl_2$	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
Oligo(dT)/ random primer or gene-specific primer per µg of RNA		250-500 ng / 50-100 ng
RNA	2-5 µg	
H_2O	Up to 50 µl	

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/µl)	1 µl	4 U/µl
5x RT Reaction buffer 2	10 µl	1 x
25 mM $MgCl_2$	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
Oligo(dT)/ random primer or gene-specific primer per µg of RNA		250-500 ng / 50-100 ng
RNA	2-5 µg	
H_2O	Up to 50 µl	

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

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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.
dNTP MIX *(20 mM of each)*	20 µmol	02-31-00020
dNTP MIX *(20 mM of each)*	100 µmol	02-31-00100
dNTP SET *(100 mM)*	4 x 25 µmol	02-21-00100
dNTP SET *(100 mM)*	4 x 100 µmol	02-21-00400

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 70^0C for 5-10 minutes, then 10-15 minutes at room temperature (for specific primer) or place in ice in case of Oligo(dT) or random primers
3. Spin for a few seconds
4. Add water
5. Add dNTPs
6. Add 5 x RT buffer 1 or

   5x RT Reaction buffer 2 and DTT and $MnCl_2$ or

   5x RT Reaction buffer 2 and DTT and $MgCl_2$
7. Add RNase inhibitor (optional)
8. Add M-MLV Reverse Transcriptase RNase H-
9. Mix gently and incubate at 37^0C for 30-90 minute

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