Data Sheet (25.08.2020)

H Minus M-MLV Reverse Transcriptase

(M–MuLV, RNase H minus)

Cat.-No.	Size	Conc.
mi-E8103	10000 units	200 units/µl

For research use only! For* in vitro *use only!

Description

H Minus M-MLV Reverse Transcriptase is a genetically modified M-MLV RT 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. The enzyme has RNA polymerization-dependent and DNA polymerization-dependent activity but lacks ribonuclease H activity.

Application

cDNA synthesis, RNA analysis by primer extension, DNA labeling.

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 37ºC.

Storage and Dilution buffer

50% glycerol (v/v), 20 mM Tris-HCl pH 7.5 at 25ºC, 200 mM NaCl, 2.5 mM DTT, 0,25 mM EDTA, 0.01% NP-40 (v/v).

Quality control

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

Protocol

Reaction volume 20 µl.

1. In a sterile microcentrifuge tube, add RNA and primer(s).
2. Add water.
3. Heat the tube at 700C for 5-10 minutes, then 10-15 minutes at room temperature (for specific primer) or place in ice in case of p(dT)12-18 or random primer.
4. Spin for a few seconds.
5. Add RT Buffer and DTT or 5 x RT Reaction Buffer.
6. Add dNTPs.
7. Add RNase inhibitor (optional).
8. Add H Minus M-MLV Reverse Transcriptase.
9. Mix gently and incubate at 37˚C for 30-90 minute.

Recommended cDNA synthesis reaction mix:

for 5 x RT Reaction buffer (with DTT).

Components	Volume	Final conc.
H Minus M-MLV RT (200 U/µl)	1 µl	10 U/µl
RT Buffer	4 µl	1 x
10 mM dNTP mix	2 µl	4 mM
100 mM DTT	0-2 µl	optional
RNase inhibitor *		optional
$p(dT)_{12-18}$ / random primer or gene-specific primer per µg of RNA		0.5-1.0µg/ 20-250ng/ 2-10 pmol
RNA / mRNA		50 ng-5 µg 100-500 ng
H_2O	Up to 20 µl	

** Although H Minus M-MLV RT DNA Polymerase is free of contaminating RNases, the use of RNase inhibitor is strongly recommended.*

*** Optional (if RNA template is GC-rich or is known to contain secondary structures). Suggest to mix RNA Primer/ RNase free H2O gently and briefly centrifuge, incubate at 65℃ for 5 min, chill on ice and briefly centrifuge, then place the tube on ice. Add other components and continue.*

Reagents Provided

1. **H Minus M-MLV Reverse Transcriptase**
2. ***5* x RT Reaction Buffer** *(with added DTT) - 100 mM Tris-HCl (pH 8.4), 250 mM KCl, 15 mM $MgCl_2$, 10 mM DTT.*

Shipping and Storage conditions

Routine storage: -20ºC

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

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.