Atlas H Minus M-MLV Reverse Transcriptase

Cat. No.	Pack Size	Conc.
BA10902	10 000 U	200 U/µl
BAS0037	1 000 U SAMPLE	200 U/µl

Application:

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

Reagents Provided:

- **Atlas H Minus M-MLV Reverse Transcriptase**
- ***5x RT Reaction Buffer*** (with added DTT) - *100 mM Tris-HCl (pH8.4), 250 mM KCl, 15 mM $MgCl_2$, 10 mM DTT.*

Description:

Atlas 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.

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.

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:

Components	Volume	Final conc.
Atlas H Minus M-MLV RT (200 U/µl)	1 µl	10 U/µl
5 x RT Buffer	4 µl	1 x
10 mM dNTP mix	2 µl	4 mM
100 mM DTT	0-2 µl	optional
RNase inhibitor *	0-1 µl	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	

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

Protocol

Reaction volume 20 µl.

1. In a sterile microcentrifuge tube, add RNA and primer(s).
2. Add water.
3. 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 $p(dT)_{12-18}$ or random primer.
4. Spin for a few seconds.
5. Add 5 x RT Reaction Buffer.
6. Add dNTPs
7. Add RNase inhibitor (optional).
8. Add Atlas H Minus M-MLV Reverse Transcriptase.
9. Mix gently and incubate at 37^0C for 30-90 minute.

Safety warnings and precautions:

For *in vitro* use only

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.