EcoNova M-MLV Reverse Transcriptase

No detectable ribonuclease H activity

Reference: AB12010; AB12011

INTENDED USE AND PRESENTATION:

EcoNova M-MLV Reverse Transcriptase (RTase) is a mutant of Moloney Murine Leukemia Virus (M-MLV) RTase with reduced RNAse H activity and increased thermal stability.

AB14000, 5000 units (25 Rxn). Concentration of enzyme 200 U/µL.

AB14001, 10000 units (50 Rxn). Concentration of enzyme 200 U/µL.

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 ηmoles of dNTPs into acid-insoluble material in 30 minutes at 74°C.

For research use only.

SUMMARY, EXPLANATION AND LIMITATIONS:

EcoNova M-MLV Reverse Transcriptase is a mutant of Moloney-Murine Leukemia Virus 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.

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

APPLICATIONS:

The EcoNova RTase is an enzyme for cDNA synthesis, RNA analysis by primer extension and DNA labeling.

PRODUCT COMPOSITION:

-EcoNova H Minus M-MLV Reverse Transcriptase
-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

METHODS AND PROCEDURE:

The reaction temperature for cDNA synthesis is 50°C and the reaction time is 60 min.

General Reaction Protocol:

The following 50 µL reaction volume can be used for cDNA synthesis.

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°C for 5-10 minutes, then 10-15 minutes at room temperature (for specific primer) or place in ice in case of $p(dT)_{25}$ or random primer.
3. Spin for a few seconds.
4. Add water.
5. Add dNTPs.
6. Add 5X 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 EcoNova H Minus M-MLV Reverse Transcriptase.
9. Mix gently and incubate at 37°C for 30-90 minute.

Recommended cDNA synthesis reaction mix:

For 5X RT Reaction buffer 1 (with $MgCl_2$ and DTT).

Components	Volume	Final conc.
EcoNova H Minus M-MLV RT (200 U/µL)	1 µL	4 U/µL
5x RT buffer 1	10 µL	1X
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
$p(dT)_{25}$ /random primer or gene-specific primer per µg of RNA	-	250-500ηg/ 50-100ηg
RNA	2-5 µg	-
H_2O	Up to 50 µL	-

Recommended cDNA synthesis reaction mixes:

For 5X RT Reaction buffer 2 (Mg^{2+} and DTT free).

Components	Volume	Final conc.
EcoNova H Minus M-MLV RT (200 U/µL)	1 µL	4 U/µL
5x RT buffer 2	10 µL	1X
20 mM $MnCl_2$	5 µL	2 mM
20 mM dNTP mix	0,5-1,25 µL	200-500 µM
100 mM DTT	2,5-5 µL	5-10 mM
RNase inhibitor *	-	optional
p(dT)25 /random primer or gene-specific primer per µg of RNA	-	250-500ηg/ 50-100ηg
RNA	2-5 µg	-
H_2O	Up to 50 µL	-

Components	Volume	Final conc.
EcoNova H Minus M-MLV RT (200 U/µL)	1 µL	4 U/µL
5x RT buffer 2	10 µL	1X
25 mM $MgCl_2$	6-12 µL	3-6 mM
20 mM dNTP mix	0,5-1,25 µL	200-500 µM
100 mM DTT	2,5-5 µL	5-10 mM
RNase inhibitor *	-	optional
p(dT)25 /random primer or gene-specific primer per µg of RNA	-	250-500ηg/ 50-100ηg
RNA	2-5 µg	-
H_2O	Up to 50 µL	-

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

REF Catalog number | LOT Batch code | RUO Research use only
Temperature limitation | Expiration date
Manufacturer | See instruction for use

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REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for PCR procedures are not provided with this polymerase. This includes sterile reaction tubes, micropipettes and tips, template DNA, dNTPs mixture, gen-specific PCR primer pair, PCR grade H_2O, heat pretreatment equipment (thermoblock, microwave), centrifuge, cold store and thermal block cycler.
Buffered solutions for DNA extraction or purification, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Gennova Scientific.

STORAGE AND STABILITY:

Store at -20°C until the expiration date printed on product label. Avoid prolonged exposure to light. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. Do not use after the expiration date. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user. The validity period of the ready to use products when opened, is the same as the expiration date indicated on the label of intact product.
Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances Gennova Scientific is obliged to cover damages caused by use of this reagent.

TROUBLESHOOTING:

If unusual amplification is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the PCR system. If this does not solve the problem, please contact Gennova Scientific's technical support department (techsupport@gennovalab.com) or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.
Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific amplification results.
Material Safety Data Sheet (MSDS) for EcoNova M-MLV RTase is available upon request.

PERFORMANCE CHARACTERISTICS:

Gennova Scientific has performed studies to evaluate the functioning of this polymerase for use with standard amplification systems, concluding that the product is both specific and sensitive for PCR performance.

BIBLIOGRAPHY:

Chien A., Edgar D.B., Trela J.M., "Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*", Journal of Bacteriology, 127(3), 1550-57, 1976.
Lawyer F.C., Stoffel S., Saiki R.K., Myambo K., Drummond R., et al., "Isolation, characterization, and expression in *Escherichia coli* of the DNA polymerase gene from Thermus aquaticus", The Journal of Biological Chemistry, 264(11), 6427-37, 1989.
Tindall K.R., Kunkel T.A., "Fidelity of DNA synthesis by the *Thermus aquaticus* DNA polymerase", Biochemistry, 27(16), 6008-13, 1988.
Innis M.A., Myambo K.B., Gelfand D.H., Brow M.A., "DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA", Proceedings of the National Academy of Sciences of the United States of America, 85(24), 9436-40, 1988.
Lo Y.M., Mehal W.Z., Fleming K.A., "Rapid production of vector-free biotinylated probes using the polymerase chain reaction", Nucleic Acids Research, 16(17), 8719, 1988.
Erlich H.A., (ed.) 1988, "PCR technology: principles and applications for DNA amplification", Stockton Press, New York.

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