GENAXXON bioscience
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M-MuLV Reverse Transcriptase

Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase (RNA-directed); EC 2.7.7.49

Component	Cat#	M3042.1010	M3042.5010	Colour code of cap
M-MuLV Reverse Transcriptase		10000 units	50000 unit	blue
Complete 5X buffer		1mL	3x 1mL	violet

Product description

The Genaxxon bioscience M-MuLV Reverse Transcriptase, encoded by Moloney Murine Leukemia Virus (M-MuLV RT) and expressed in *E.coli* is a RNA-dependent DNA polymerase that synthesizes the cDNA first strand from a single-stranded RNA template to which a primer has been hybridized. M-MuLV RT will also extend primers hybridized to single-stranded DNA! Second strand cDNA synthesis can be achieved from some mRNA templates without an additional DNA polymerase.

This enzyme has been genetically altered to remove the associated RNase H acitivity. Removal of RNase H activity resulted in an increase of full-length cDNA.

Supplied buffers/solutions

M-MuLV:	200 units/µL in storage buffer* containing 50% glycerol (v/v).
5X Reaction Buffer:	250mM Tris/HCl (pH8.3), 375mM KCl, 15mM MgCl2, 50mM DTT
* Storage buffer:	200mM potassium phosphate, pH7.2, 2mM DTT, 0.2% Triton X-100, 50% glycerol

Unit definition

One unit of M-MuLV RT is defined as the amount of enzyme that incorporates 1nmol of dTTP's into acid-insoluble fraction in 10 minutes at 37°C using poly(A) oligo dT as a template primer.

Quality Control

Nuclease activity: 50ng of radio labelled DNA or RNA is incubated with 200 units of the enzyme in 1X reaction buffer for one hour at 37°C, resulting in <1% release of free measurable radio activity in the supernatant.

Endonuclease activity: 1µg of Type 1 supercoiled plasmid DNA is incubated with 500 units of enzyme in 1X reaction buffer for one hour at 37°C. The supercoiled DNA is visualized on an ethidium bromide stained agarose gel to verify absence of nicking or cutting activity.

Purity: >90% judged by SDS-polyacrylamide gel. M-MuLV is free of detectable RNase, and DNase (exo- and endonuclease) activity.

Stability and Storage

M-MuLV RT, including buffers and reagents, should be stored immediately upon receipt at -20°C.
When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
For more information, please consult the appropriate material safety data sheets (MSDSs).
These are available online as pdf-file or on request (info@genaxxon.com).

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Application

RT PCR
Synthesis of cDNA
mRNA 5'-end Mapping by Primer Extension Analysis
End-labeling of DNA
Dideoxynucleotide Sequencing

Product Use Limitations

The Genaxxon biosience M-MuLV is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety information

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

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RT Protocol Part

Important notes before getting started

Things to do before starting

- **Thaw 5X buffer, dNTPs or dNTP-mix, primer solutions** at RT or on ice.
  Keep the solutions on ice after complete thawing.
  Mix well before use to avoid localized differences in salt concentration.

First Step Protocol - RT- Reverse Transcription / cDNA synthesis for standard RNA

Add the below mentioned components to a nuclease-free microtube. Pipett on ice and mix the components by pipetting gently up and down.
Note: In general, water, RNA and primers should be mixed together before the remaining components are added.

Mix-1: Assay preparation (without sample denaturation) - for standard RNA/primer combinations only.

Component	Stock conc.	Final conc.	20µL assay
RNase-free water	-	-	fill up to 20µL
RNA template	-	Total RNA: 10pg up to 5µg or mRNA: 10pg up to 500ng	x µL
Primer	10µM	-gene-specific primer: 10 - 20 pmol (50ng - 100ng) -oligo-dT20 primer: 50pmol (300ng); cat#: M3039 -random primer: 50pmol (100ng); cat#: M3038	1µL - 2µL 5µL 5µL
M-MuLV Buffer complete	5X	1X	4µL
dNTP-Mix	10mM each	500µM each	1µL
DTT stock solution [1)]	100mM	5mM	1µL
RNase Inhibitor [2)]	40 units/µL	20 - 40 units (cat#: M3034)	0.5µL - 1µL
M-MuLV [3)]	200 units/µL	100 - 200 units	0.5µL - 1µL

1) Adding of up to 5mM DTT may increase the yield and is recommended for indivicual optimization.
2) Addition of 20 – 40 units RNase Inhibitor is recommended and may be essential if working with low amounts of starting RNA.
3) 100 units of enzyme is recommended for standard assays but increasing the amount of enzyme to 200 units may result in even higher transcription yields under selected assay conditions.

The Genaxxon M-MuLV together with the supplied buffer and salt solutions are free of RNase activity, meanwhile we recommend to add RNase Inhibitor into the mixture to inhibit possible RNase contaminations of the sample.

cDNA synthesis protocol using gene specific primers

Step	Time	Temperature
cDNA synthesis	30 – 120 min [4)]	50°C [5)]
Inactivation of M-MuLV	10 min	65°C to 70°C

cDNA synthesis protocol using oligo-dT or random primers

Step	Time	Temperature
cDNA synthesis	10 min	42°C
cDNA synthesis	30 – 120 min [4)]	50°C [5)]
Inactivation of M-MuLV	10 min	65°C to 70°C

4) The optimal time depends on the length of cDNA. Incubation of 30 min for cDNA with 500bp. 120 min for cDNA with >1.5 kbp length.
5) The optimal temperature depends on the structural features of the RNA. Increase the temperature to 55 °C for difficult templates with high secondary structure.

Note: The optimal reaction time and temperature should be adjusted for each particular RNA.

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First Step Protocol - RT- Reverse Transcription / cDNA synthesis for RNA with high degree of secondary structure.

Add the below mentioned components to a nuclease-free microtube.
Pipett on ice and mix the components by pipetting gently up and down.

Mix 1 / denaturation mixture:
Assay preparation (with sample denaturation) - for RNA/primer with a high degree of secondary structure.

Component	Stock conc.	Final conc.	20µL assay
RNase-free water	-	-	fill up to 10µL
RNA template	-	Total RNA: 10pg up to 5µg or mRNA: 10pg up to 500ng	x µL
Primer	10µM	-gene-specific primer: 10 - 20 pmol (50ng - 100ng) -oligo-dT20 primer: 50pmol (300ng); cat#: M3039 -random primer: 50pmol (100ng); cat#: M3038	1µL - 2µL 5µL 5µL

Denaturation and primer annealing

Incubate the mixture at 65°C to 70°C for 5 to 10 min and place it at room temperature (if using specific primer) or on ice (if using oligo-dT or random primer).

Incubation	Temperature
5 min to 10 min	65°C to 70°C
10 - 15 min for specific primers **or** 5 min for Oligo dT / random primer	room temperature place on ice

Preparation of the Reaction Mix

Add the following components to a further nuclease-free microtube and mix by pipetting gently up and down:

Mix 2 / reaction mixture:
Assay preparation (with sample denaturation) - for RNA/primer with a high degree of secondary structure.

Component	Stock conc.	Final conc.	20µL assay
RNase-free water	-	-	fill up to 10µL
M-MuLV Buffer complete	5X	1X	4µL
dNTP-Mix	10mM each	500µM each	1µL
DTT stock solution [1)]	100mM	5mM	1µL
RNase Inhibitor [2)]	40 units/µL	20 - 40 units (cat#: M3034)	0.5µL - 1µL
M-MuLV [3)]	200 units/µL	100 - 200 units	0.5µL - 1µL

1) Adding of up to 5mM DTT may increase the yield and is recommended for individual optimization.
2) Addition of 20 - 40 units RNase Inhibitor is recommended and may be essential if working with low amounts of starting RNA.
3) 100 units of enzyme is recommended for standard assays but increasing the amount of enzyme to 200 units may result in even higher transcription yields under selected assay conditions.

The Genaxxon M-MuLV together with the supplied buffer and salt solutions are free of RNase activity, meanwhile we recommend to add RNase Inhibitor into the mixture to inhibit possible RNase contaminations of the sample.

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