Lucigen®
Simplifying Genomics

NxGen® M-MuLV Reverse Transcriptase

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support

Email: techserv@lucigen.com

Phone: (888) 575-9695

Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Product Description

M-MuLV Reverse Transcriptase is an RNA-dependent DNA polymerase which shows no measurable 3′→5′ proofreading function. This enzyme can copy a single-stranded DNA template or perform cDNA synthesis by extending a DNA primer annealed to an RNA template.

Storage buffer: M-MuLV Reverse Transcriptase is supplied in 200,000 U/mL in 50 mM Tris-HCl, 150 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% NP-40 Alternative, 50% glycerol, pH 7.6 @ 25 °C.

10X M-MuLV RT Buffer: 500 mM Tris-HCl, 750 mM KCl, 30 mM MgCl2, 100 mM dithiothreitol, pH 8.3 @ 25 °C.

Source: A recombinant E. coli strain carrying the Moloney-Murine Leukemia Virus Reverse Transcriptase gene.

Unit Definition: 1 unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid insoluble material in 10 minutes at 37 °C using poly r(A)/oligo (dT) as a substrate.

Product Specifications

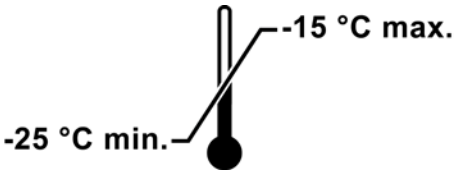
TEST	SPECIFICATION
Unit Concentration	200,000 U/mL
Purity (SDS-PAGE)	>99%
SS Exonuclease	200 U <5.0% released
DS Exonuclease	200 U <0.5% released
Endonuclease	200 U <10% converted
E.coli 16S rDNA Contamination	200 U <10 copies

Product Designations

Product	Kit Size	Catalog number
NxGen® M-MuLV Reverse Transcriptase	50,000 Units	30222-1
	250,000 Units	30222-2

Components & Storage Conditions

Store all Kits and Components at -20 ºC



The NxGen® M-MuLV package consists of the following components:

Description	Part Number	50,000 U	250,000 U
NxGen M-MuLV Reverse Transcriptase	F83902-1	250 µL	5 x 250 µL
10X M-MuLV RT Buffer*	F88903-1	1.5 mL	5 x 1.5 mL

*Avoid excessive freeze-thaw of the 10X M-MuLV RT Buffer. Repeated freeze-thaw may lead to buffer precipitation. If precipitation occurs, warm the buffer at 37 °C for 10 minutes prior to use. The buffer may be stored at 4 °C.

First Strand Reaction Protocol

General precaution against RNAse degradation of template RNA should be taken when setting up First-Strand reactions. Use nuclease-free water, RNase inihibitor, RNAse-free tubes, and sterile pipet tips with filters. The following procedure can be used as a general guideline for preparing a 10 µL reaction.

1. In a sterile microcentrifuge tube, add the following components on ice:

Volume, µL	Component	Final Concentration/ Amount*
2.0	10 mM dNTP Mix	2.0 mM
X	Total RNA **-or-** polyA-selected mRNA	1ng – 2 µg 5 – 500 ng
1	Oligo $(dT)_{12-18}$ **-or-** Random Hexamers (125 µg/mL) **-or-** Gene-Specific Primers (2 pmol)	40 µg/mL 10 µg/mL 165 µM
Bring to 8 µL	Nuclease-free water	N/A

*based on 10 µL total volume

2. Heat the reaction for 5 minutes at 65 °C. Spin briefly (5 seconds) to collect condensate. Place the tube immediately on ice.
3. Add 1 µL 10X RT Buffer. Mix by gently pipetting.
4. Incubate:
   a. 2 minutes at 42 °C if using Oligo (dT) or gene-specific primers; **-or-**
   b. 2 minutes at 25 °C if using random hexamers.
5. Add 1 µL (200 U) M-MuLV Reverse Transcriptase for a total reaction volume of 10 µL. Mix by gently pipetting.
   (Note: If using random hexamers, incubate the reaction at 25 °C for 10 minutes, then proceed to step 6.)

6. Incubate the reaction at 42 °C for 45 – 60 minutes.
7. Inactivate the enzyme by incubating at 85 °C for 10 minutes.
8. Store products at -20 °C or proceed to next step.

References

1. Engler, M.J. and Richardson, C.C. (1982) P.D. Boyer (Eds.), The Enzymes, 5, pp. 3. San Diego: Academic Press.

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