

Manual

NxGen M-MuLV Reverse Transcriptase

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT
-20 °C storage required
immediately upon receipt

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NxGen M-MuLV Reverse Transcriptase

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NxGen M-MuLV Reverse Transcriptase

1. Product description

NxGen™ 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 units/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: One 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.

2. Product specifications

Test	Specification
Unit concentration	200,000 units/mL
Purity (SDS-PAGE)	>99%
SS exonuclease	200 units <5.0% released
DS exonuclease	200 units <0.5% released
Endonuclease	200 units <10% converted
<i>E. coli</i> 16S rDNA Contamination	200 units <10 copies

3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
NxGen M-MuLV Reverse Transcriptase	50,000 units	30222-1	NxGen M-MuLV Reverse Transcriptase	F83902-1	250 µL
			10X M-MuLV RT Buffer*	F88903-1	1.5 mL

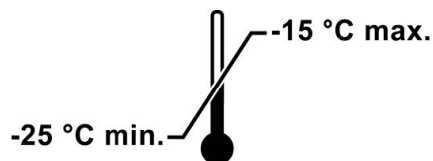
* Avoid excessive freeze-thaw of the 10X M-MuLV RT Buffer. Repeated freeze-thaw may lead to buffer precipitation. If precipitation occurs, warm the buffer at 37 °C for 10 minutes prior to use. The buffer may be stored at 4 °C.

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4. Storage conditions

Store all kits and components at -20 °C.



5. First strand reaction protocol

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

	Volume	Final concentration/amount**
10 mM dNTP Mix	2 µL	2.0 mM
Total RNA -or-	X µL	1 ng-2 µg
polyA-selected mRNA	2 µL	5-500 ng
Oligo (dT) ₁₂₋₁₈ -or-	1 µL	40 µg/mL
Random hexamers (125 µg/mL) -or-	1 µL	10 µg/mL
Gene-specific primers (2 pmol)	1 µL	165 µM
Nuclease-free water	Bring to 8 µL	N/A

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 M-MuLV 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 units) 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.

6. Reference

1. Engler MJ and Richardson CC 1982 Boyer PD (Ed.), The Enzymes, 5, pp. 3. San Diego: Academic Press.

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7. Technical support and guarantee

LGC, Biosearch Technologies™ 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 usersupplied reagents are of high 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.

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Product guarantee: Biosearch Technologies 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 date of receipt.



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