



Product Information

M-MuLV Reverse Transcriptase

Part Number:	P704L
Lot Number:	

Rev.D

Product Description:

M-MuLV Reverse Transcriptase is a DNA polymerase which utilizes RNA as a substrate and exhibits no measurable proofreading 3'→5' exonuclease function. This enzyme can perform cDNA synthesis by extending off a DNA primer annealed to an RNA template, or can copy a single-stranded DNA template.

Source of Protein

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

Supplied 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

Supplied With

B704 (10X M MuLV RT Buffer)

10X M-MuLV RT Buffer (B704)

500 mM Tris-HCl
750 mM KCl
30 mM MgCl₂
100 mM dithiothreitol
pH 8.3 @ 25°C

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 Specification*

Unit Size:	100,000
Unit Concentration	200,000 U/mL
Protein Concentration	0.85 mg/mL
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	235,300 U/mg
SS Exonuclease	14,000 U <0.1% released
DS Exonuclease	14,000 U <0.1% released
Endonuclease	14,000 U <10% converted
<i>E. coli</i> 16S rDNA Contamination	14,000 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X M-MuLV RT Buffer ([RT]_f = 0.002-0.00002 μg/μL) and added to 50 μL reactions containing 20 μg poly r(A) DNA, 10 μg oligo (dT), 1X RT Buffer, 4mCi/mL ³H-dTTP and 250 μM dTTP. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (*Molecular Cloning, v3, 2001, pp. A8.25-A8.26*).

SDS-Page (Physical Purity Assessment)

2.0 μL of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 μL of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Protein Concentration (OD₂₈₀) Measurement

A 3.0 μL sample of enzyme was analyzed at OD₂₈₀ using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 101,420 and molecular weight of 74,559 Daltons. Acceptance for this assay is +/- 5% of reference sample.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 μ L reaction containing 15,000 cpm of a radiolabeled single-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 μ L reaction containing 15,000 cpm of a radiolabeled double-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Endonuclease Activity

A 50 μ L reaction containing 1 μ g of pBR322 DNA and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

***E. coli* 16S rDNA Contamination Test**

Replicate 5 μ L samples of enzyme solution were heat denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using primers for the 16S rRNA locus. Based on no template control C_t values, the detection limit of this assay is <10 copies genome/sample.

Usage Protocol (First Strand Synthesis):

1. **Primer Annealing:** Combine the following in an RNase-free reaction vessel:

Amount	Description	Final Concentration
1 μ L	25mM dNTP Solution (N205L)	2.0 mM
X μ L	1ng-2 μ g Total RNA -or-	
X μ L	5-500 ng mRNA (polyA selected)	
1 μ L	Oligo (dT) ₁₂₋₁₈ (500 μ g/ml) -or -	40 μ g/ml
1 μ L	Random Primers (125 μ g/ml) -or	10 μ g/ml
1 μ L	GSP Primer (2 pmol)	165 μ M
X μ L	Sterile, Type I Water	N/A
12 μL	Total Volume	

- Heat reaction for 5 minutes at 65°C. Spin briefly (5 sec) to pull down condensate and place immediately on ice.
- Add 1 μ L 10X M-MuLV RT Buffer (B704) and 7 μ L Type I Water to reaction.
- Incubate:
 - If using Oligo (dT) or GSP primers: 2 minutes @ 42°C
 - If using Random primers: 2 minutes @ 25°C
- Add 1 μ L (200 units) M-MuLV Reverse Transcriptase (P704) and mix by gently pipetting sample. (Note: if using random primers, pre-incubate reaction @25°C for 10 minutes).
- Incubate at 42°C for 45-60 minutes.
- Inactivate enzyme at 85°C for 10 minutes.
- Store products at -20°C or proceed to next step.



Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.