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**Product Information Sheet** *P7040L Rev F*

| Product Information | |
|---|---|
| **M-MuLV Reverse Transcriptase** | |
| **Part Number** | P7040L 280550 |
| **Concentration** | 200,000 U/mL |
| **Unit Size** | 100,000 U |

| Product Specification | |
|---|---|
| **Storage Temperature** | -25°C to -15°C |
| **TEST:** | **SPECIFICATION:** |
| **Purity (SDS-PAGE)** | >99% |
| **Specific Activity** | 169,000 U/mg |
| **SS Exonuclease** | 2,000 U <5.0% released |
| **DS Exonuclease** | 2,000 U <1.0% released |
| **DS Endonuclease** | 2,000 U =No conversion |
| ***E.coli* DNA Contamination** | 2,000 U <10 copies |
| **RNAse Contamination** | 2,000 U = No Detectable non-specific RNase |

## 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 DTT
0.1% NP-40 Alternative
50% glycerol
pH 7.6 @ 25°C

### Supplied with

B7040 (10X M MuLV RT Buffer)

### 10X M-MuLV RT Buffer (B7040):

500 mM Tris-HCl
750 mM KCl
30 mM $MgCl_2$
100 mM DTT
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.

## 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 and added to 50 µL reactions containing 20 µg/mL poly r(A) RNA, oligo (dT) DNA, 1X RT Buffer, $^{3}$H-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*).

### Protein Concentration ($OD_{280}$) Measurement

Protein concentration is determined utilizing Bio-Rad Protein Assay Kit II (500-0002). Three replicate 5.0uL samples of enzyme were tested at concentrate, 1:10 and 1:100 dilutions. Absorbance at 595nm and comparison to a BSA standard curve in the 1-10ug range allows for relative protein determination.

### 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

## Contamination Tests:

### Single-Stranded Exonuclease Activity

A 50 µL reaction containing 10,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 5.0% release of TCA-soluble counts.

*(additional information on reverse side)*

### Double-Stranded Exonuclease Activity

A 50 µL reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 1.0% release of TCA-soluble counts.

### Double-Stranded Endonuclease Activity

A 50 µL reaction containing 0.5 µg of pBR322 DNA and 10 µL 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 5uL samples of enzyme solution were denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus. The acceptance criterion for the test is the threshold cycle count ($C_t$) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control $C_t$ values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

### Non-Specific RNAse Assay

Replicate 10 uL samples were screened for non-specific RNAse contamination using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines

## Usage Instructions:

### First Strand Synthesis

| Amount | Description | Final Concentration |
|---|---|---|
| 1 µL | 25mM dNTP Solution (N2050L) | 2.0 mM |
| X µL | 1ng-2µg Total RNA ***–or-*** | |
| X µL | 5-500 ng mRNA (polyA selected) | |
| 1 µL | Oligo $(dT)_{12\text{-}18}$ (500 µg/ml) ***–or -*** | 40 µg/ml |
| 1 µL | Random Primers (125 µg/ml) ***-or*** | 10 µg/ml |
| 1 µL | GSP Primer (2 pmol) | 165 µM |
| X µL | Sterile, Type I Water | N/A |
| **10 µL** | **Total Volume** | |

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

2. Heat reaction for 5 minutes at 65°C. Spin briefly (5 sec) to pull down condensate and place immediately on ice.
3. Add 1 µL 10X M-MuLV RT Buffer (B7040) and Type I Water to a final volume of 10 µL per reaction.
4. Incubate:
   a. If using Oligo (dT) or GSP primers: 2 minutes @ 42°C
   b. If using Random primers: 2 minutes @ 25°C
5. Add 1 µL (200 units) M-MuLV Reverse Transcriptase (P7040) and mix by gently pipetting sample. (Note: if using random primers, pre-incubate reaction @25°C for 10 minutes).
6. Incubate at 42°C for 45-60 minutes.
7. Inactivate enzyme at 85°C for 10 minutes.
8. Store products at -20°C or proceed to next step.

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### 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.