**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.

### 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.

### Molecular weight
68,100 Daltons

### Quality Control Analysis

**Unit Activity** is 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, 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).

**Protein Concentration** is determined using the Bio-Rad Protein Assay Kit II (500-0002).

**Physical Purity** is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest bands in the diluted sample.

**Single-Stranded Exonuclease** is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

**Double-Stranded Exonuclease** is determined in a 50 µL reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

**Double-Stranded Endonuclease** is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.
**Product Specifications P7040L Rev F**

**E.coli 16S rDNA Contamination** is evaluated using 5 µL replicate samples of enzyme solution 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.

**Non-Specific RNAse** contamination is assessed using the RNase Alert kit, (Integrated DNA Technologies), following the manufacturer’s guidelines.

**Supplied in:** 50mM Tris-HCl, 150mM NaCl, 1mM DTT, 0.1mM EDTA, 0.1% NP-40 Alternative, 50% glycerol pH 7.6 @ 25°C.

**Supplied with:**
10X M-MuLV RT Buffer (B7040): 500mM Tris-HCl, 750mM KCl, 30mM MgCl₂, 100mM DTT pH 8.3 @ 25°C.

**Usage Instructions:**

**First Strand Synthesis**

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

<table>
<thead>
<tr>
<th>Amount</th>
<th>Description</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µL</td>
<td>25mM dNTP Solution (N2050L)</td>
<td>2.0 mM</td>
</tr>
<tr>
<td>X µL</td>
<td>1ng-2µg Total RNA –or-</td>
<td></td>
</tr>
<tr>
<td>X µL</td>
<td>5-500 ng mRNA (polyA selected)</td>
<td></td>
</tr>
<tr>
<td>1 µL</td>
<td>Oligo (dT)₁₂₋₁₈ (500 µg/ml) –or-</td>
<td>40 µg/ml</td>
</tr>
<tr>
<td>1 µL</td>
<td>Random Primers (125 µg/ml) –or-</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>1 µL</td>
<td>GSP Primer (2 pmol)</td>
<td>165 µM</td>
</tr>
<tr>
<td>X µL</td>
<td>Sterile, Type I Water</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Total Volume**

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

**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. SDS sheets relevant to this product are available upon request.

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