

# M-MLV Reverse Transcriptase

Store at  $-20^{\circ}\text{C}$ .  
Do not store in a frost-free freezer.

<b>Catalog # (P/N):</b>	AM2043	AM2044
<b>Amount:</b>	4,000 Units	10,000 Units
<b>Source:</b>	An <i>E. coli</i> strain overexpressing an engineered version of the <i>pol</i> gene of M-MLV.	
<b>Unit Concentration:</b>	100 U/ $\mu\text{L}$	
<b>Unit Definition:</b>	One unit of M-MLV Reverse Transcriptase incorporates 1 nmol dTTP into acid-precipitable material in 10 min at $37^{\circ}\text{C}$ , using poly(A):oligo(dT) as template:primer.	
<b>Additional Materials Included:</b>	500 $\mu\text{L}$ 10X First Strand Synthesis Buffer 500 mM Tris pH 8.3 750 mM KCL 50 mM DTT 30 mM $\text{MgCl}_2$	
<b>Storage Conditions:</b>	Store at $-20^{\circ}\text{C}$ . <b>Do not store in a frost-free freezer.</b>	
<b>Storage Buffer:</b>	(Not included) 20 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.05% Triton X-100, 0.1 mM $\text{Na}_2\text{EDTA}$ , 0.1 mM NaCl, 50% (v/v) glycerol.	

## USER INFORMATION

**Product Description:** Ambion M-MLV Reverse Transcriptase (RT) is derived from a cloned region of the *pol* gene of M-MLV and isolated from an *E. coli* strain overexpressing this construct. In the presence of a single-stranded RNA or DNA template, primer and deoxynucleotides, this enzyme will extend the oligonucleotide primer, producing a complementary strand. M-MLV Reverse Transcriptase is commonly used in the first step of RT-PCR as well as for primer extension experiments. A 10X First Strand Synthesis Buffer is provided with the enzyme.

### Applications:

#### Reverse Transcription of RNA (First Strand Synthesis)

- Mix RNA and primers and heat
  - 1–2  $\mu\text{g}$  total RNA, or 20–100 ng poly(A) RNA.
  - 2  $\mu\text{L}$  of 50  $\mu\text{M}$  oligo(dT) or random decamers, or equivalent.
  - Add nuclease-free water to achieve a volume of 12  $\mu\text{L}$ .
- Mix, spin briefly, heat 3 min at  $70\text{--}85^{\circ}\text{C}$ .
- Remove tube to ice; spin briefly and replace on ice.
- Add remaining components (final volume 20  $\mu\text{L}$ )
  - 2  $\mu\text{L}$  10X First Strand Synthesis Buffer (included).
  - 4  $\mu\text{L}$  dNTP mix, 2.5 mM each.
  - 1  $\mu\text{L}$  RNase Inhibitor (P/N AM2682, AM2684; 40 U/ $\mu\text{L}$ ).
  - 1  $\mu\text{L}$  M-MLV Reverse Transcriptase, 100 U/ $\mu\text{L}$ .
- Mix gently, spin briefly.
- Incubate at  $42\text{--}44^{\circ}\text{C}$  for 1 hr.
- Incubate at  $92^{\circ}\text{C}$  for 10 min to inactivate the M-MLV Reverse Transcriptase.

For further information, see the Instruction Manual for the Ambion RETROscript<sup>®</sup> Kit (P/N AM1710) available at: [www.ambion.com](http://www.ambion.com)

## QUALITY CONTROL

M-MLV Reverse Transcriptase is thoroughly tested for contaminating nonspecific endonuclease, exonuclease, RNase, and protease activity. Functionality is determined using a standard RETROscript<sup>®</sup> Kit (P/N AM1710) assay for amplification with positive control primers.

## OTHER INFORMATION

### Material Safety Data Sheets:

Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: [www.ambion.com/techlib/msds](http://www.ambion.com/techlib/msds). Alternatively, e-mail your request to [MSDS\\_Inquiry\\_CCRM@appliedbiosystems.com](mailto:MSDS_Inquiry_CCRM@appliedbiosystems.com). Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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