AMID BIOSCIENCES

Protein Engineering Company

**Product Information**
**Product name:** M-MLV Reverse Transcriptase
**Catalog number:** MRT-301

**Description**
Amid Biosciences Reverse Transcriptase is an engineered version of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) with a minimum RNase H activity and enhanced thermostability compared to wild-type M-MLV RT. The enzyme can copy a single-stranded DNA template or perform cDNA synthesis by initiating from a primer annealed to an RNA template. The optimal fist-strand cDNA synthesis temperature for this enzyme is 42°C, however, the enzyme is active up to 50º C, providing increased specificity, higher yields of cDNA, and more full-length product. It can generate cDNA up to 12 kb
The enzyme is isolated from an E. coli strain overexpressing M-MLV RT gene construct.

**Size:** 10,000 Units
**Concentration**: 200 u/µl

**Storage Buffer**
20 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.05% (v/v) Triton X-100, 0.1 mM EDTA, 0.1 M NaCl and 50% (v/v) glycerol.

**Reaction Buffer (5x)**
250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$

**Unit Definition**
One unit of the enzyme incorporates 1 nmole of dTTP into acid-precipitable material in 10 minutes at 37˚C using poly (A):oligo $(dT)_{25}$ as template-primer.

**Quality Control**
This enzyme has passed the quality control assays: SDS-PAGE analysis for purity, functional absence of endonuclease activities, functional absence of exonuclease activities, functional absence of protease activity.

**Storage and Handling:** -20° C

**Usage:** For research use only. Not intended for any animal or human therapeutic or diagnostic use.

| **Product Components** | **Volume** |
|---|---|
| M-MLV Reverse Transcriptase (200 U/µL) | 50 µL |
| 5X Reaction Buffer (250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$) | 250 µL |
| 0.1 M DTT | 100 µL |

**Protocol for Reverse Transcription of RNA (First Strand Synthesis)**
**Prepare the following mixture in a 0.2 mL nuclease-free tube.**

| **Component** | **Volume** |
|---|---|
| 50 µM oligo(dT)20 primer, 50 µM Random 6mers, or 2 µM gene-specific reverse primer | 1 µL (2 µL for Random 6-mers) |
| 10 mM dNTP mix (10 mM each) | 1 µL |
| Template RNA | 10 pg–5 µg total RNA or 10 pg– 500 ng mRNA |
| RNase Free $dH_2O$ | to 12 µL |

**Mix, spin briefly and heat for 5 min at 65° C, and then incubate immediately on ice.**
**Collect the contents of the tube by brief centrifugation and add remaining components (final volume 20 µL).**

| **Component** | **Volume** |
|---|---|
| Template RNA Primer Mixture (from step 2) | 12 µL |
| 5X Reaction Buffer | 4 µL |
| 0.1 M DTT | 2 µL |
| RNase Inhibitor (40 Units/ µL) (optional) | 1 µL |
| M-MLV Reverse Transcriptase | 1 µL |

**Incubate reactions**:

1. If using oligo(dT)20 or gene-specific primer, directly proceed to step 3
2. If using random 6-mers, incubate the combined reaction mixture at 23°C for 10 min, and then proceed to step 3.
3. Mix gently, spin briefly, and incubate at 42°C for 30-60 min.

**Note:** In general, reactions should be performed at 42°C. However, for RT-PCR reactions where the gene specific primer is used for cDNA synthesis, we recommend performing the reverse transcription reaction at 50°C to reduce the possibility of non-specific amplification products.

**Inactivate reactions:**

1. Incubate at 92°C for 10 min to inactivate the M-MLV Reverse Transcriptase, then cool on ice.
2. For synthesis of longer cDNAs (> 4 kb), inactivation at 70°C for 15 min is recommended to minimize cDNA damage.

The cDNA can be used as a template for amplification in PCR. However, amplification of some PCR targets (>1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 µL (2 units) of E. coli RNase H and incubate at 37°C for 20 min.
Use only 10% of the first-strand reaction for PCR. Higher volumes may not increase amplification and may result in decreased amounts of PCR product.