CERTIFICATE OF ANALYSIS

Product Name:	**M-MLV Reverse Transcriptase (200U/μL)**
Cat. No.:	PBIMV0030
Lot. No.:	
Date of Manufacture:	
Expiration Date:	
Unit definition:	One unit (U) is defined as the amount of enzyme that incorporates 1nmole of dTTPs into acid precipitable material in 10 minutes at 37°C with Oligo$(dT)_{20}$-poly(rA) as the primer / template.
Concentration:	200 U/μL
Quantity	10,000 Units
Source:	Recombinant *E. coli* strain.
Optimal Storage Temp:	-20°C ± 5°C
Storage buffer:	20 mM Tris-HCl (pH 7.6), 300 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 0.1% NP-40, 50% glycerol.

Parameter	Specifications	Results
Appearance	Clear, colorless	Corresponds
Specific activity	≧ 100,000 U/mg	Corresponds
E.coli genomic DNA Contamination	≦ 0.1 pg/200 U	Corresponds
Ribonucleases Activity	Not detectable	Corresponds
Non-Specific DNase Activity	Not detectable	Corresponds
Protein Purity	≧ 90%	Corresponds

Signature of Quality Assurance Supervisor, Date

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Quality Control Analysis

- ✓ ***E.coli* genomic DNA Contamination:** 200 units M-MLV Reverse Transcriptase denatured and assessed using QuantStudio Absolute Q Digital PCR System, (ThermoFisher), for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

- ✓ **Ribonucleases Activity:** To test the presence of *E.coli* RNase activity, 1μg of MS2 RNA(Bacteriophage) is incubated with 200 units of M-MLV Reverse Transcriptase for 1 hour at 37°C,and the RNA is then visualized on a SYBR™ Gold-stained agarose gel to verify the absence of degradation.

- ✓ **Non-Specific DNase activity:** 1 μg of Lambda-HindIII is incubated with 100 units of M-MLV Reverse Transcriptase for 16 hours at 37°C, and the DNA pattern is then visualized on an ethidium bromide-stained agarose gel to verify the absence of degradation.

- ✓ **Protein Purity Assay:** M-MLV Reverse Transcriptase is ≧ 90% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Product Information

I. List of Components

Store all components at -20℃.

- MMLV Reverse Transcriptase.
- 5X Reverse Transcription Buffer

II. Additional Materials Required

- Ribonuclease Inhibitor
- dNTP Mix (10 mM each)
- RNase-free H_2O
- Oligo $(dT)_{12-18}$ primer (50 µM) or random primers (25 µM) or specific primer (10 uM)
- 1M DTT

III. Procedure for Routine First-Strand cDNA Synthesis Reactions

1. Add the following components to a nuclease-free microcentrifuge tube:

Component	Volume	
Oligo (dT)12-18 primer (50 µM) or Random primers (25 µM) or Specific primer (10 µM)	2	µl
10 mM dNTPs (each)	1	µl
Template RNA*	Up to 1	µg
RNase-free H_2O	Up to 10	µl

* 1 ng-1 µg total RNA or 50 pg-100 ng poly(A)-RNA

2. Heat mixture to 70°C for 5 minutes to melt secondary structure within the template.
3. Cool the tube immediately on ice to prevent secondary structure from reforming, then spin briefly to collect the solution at the bottom of the tube.
4. Add the following components to the tube by brief centrifugation and add:

Component		Volume
5X M-MLV Reaction Buffer	4	µl
DTT, 1M	0.2	µl
Ribonuclease Inhibitor (20 U/µl)	1	µl
M-MLV RT (200 U/µl)	1	µl
RNase-free H_2O	Up to 20	µl

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5. Incubate the 20 µl cDNA synthesis reaction at 45°C*AA for 30-60*B minutes. If random primer is used, an incubation step at 25°C for 10 minutes is recommended before the 45°C incubation.

*Note:

A. M-MLV Reverse transcriptase can be used at 37 - 55°C. It's generally recommended to perform the RT reaction at 45°C. If the reverse primer for PCR is also used as a primer, non-specific products may be amplified due to mispriming. In such a case, perform RT reaction at 50 - 55°C for 30 minutes.

B. In most cases, 30 minutes is sufficient. Increase the incubation time to 60 minutes when the target is very long.

6. Inactivate the enzyme at 70°C for 10 minutes then cool on ice. The cDNA product should be stored at -20 ± 5°C and can be used for 2nd-strand cDNA synthesis or as a template for PCR.