WizPure™ MMLV RTase (RNase H+)

- W2301 **10,000 U**
- W2301-5 **50,000 U**

Description
WizPure™ MMLV RTase (RNase H+) encoded by Moloney Murine Leukemia Virus (MMLV RT) is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template to which a primer has been hybridized. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA or DNA templates. MMLV RTase will also extend primers hybridized to single-stranded DNA. Second strand cDNA synthesis can be achieved from some mRNA templates without an additional DNA polymerase.

Kit Contents

Contents	W2301	W2301-5
MMLV RTase (RNase H+), (200U/µl)	10,000 U	50,000 U
5X Reaction Buffer	500 µl	2,500 µl

Storage Buffer
20 mM Tris-HCl, 200 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.01% NP-40, 50% glycerol, pH 7.5 (25°C).

5X Reaction Buffer
250 mM Tris-HCl, 375 mM KCl, 15 mM MgCl2, 50 mM dithiothreitol, 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.

Storage Conditions
• Store all components at -20ºC in a non-frost-free freezer.

cDNA synthesis protocol
1. mix 1-5 µg RNA with
   1 µl of 100pmol/µl oligo-dT15-25 or random primers,
   or 1 µl of 10pmol/µl gene-specific primer per µg of RNA.

2. Incubate the mixture at 70 °C for 5-10 min
3. Cooling on ice
4. Add the following components in the order listed to a PCR tube

Components	Volume	Final conc.
MMLV RTase (RNase H+), (200 U/µl)	1 µl	10 U
5X Reaction buffer	4 µl	1 X
2.5 mM dNTP mix	4 µl	200-500 µM
RNase inhibitor (40 U/µl)	0.5 µl	* optional
ddH_2O	Up to 20 µl	

5. Mix well and spin briefly.
6. Incubate at 42~50ºC for 30~60 min.
7. Incubate at 95ºC for 5 min. for reaction stop.
8. Store products at -20°C or proceed to next step.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity
A 50 µl reaction containing 11,000 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.

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 37ºC resulted in less than 0.5% release of TCA-soluble counts.

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 5 µl 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 (Ct) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control Ct values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

Quality Control Analysis:

Unit Characterization Assay
Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer. Reactions were incubated 10 minutes at 75°C, plunged on ice, and analyzed using the method of Sambrook and Russell.

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.

Quality Authorized by : Jamie Ahn 

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