Product Information

*Pyro*RTase™

Catalog No.: 40501
Product Name: *Pyro*RTase™

Description

*Pyro*RTase™, a thermostable M-MLV Reverse Transcriptase, is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template with hybridized primer. *Pyro*RTase™ is purified from RNaseH mutant *E. coli* and shows 100% activity at 50°C.

Unit definition

One unit of *Pyro*RTase™ incorporates 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C.

Concentration	Package size	Storage
100 U/μL	5 KU/set	-20°C

Storage buffer

20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.25 mM EDTA, 0.01% NP-40(v/v), 2.5 mM DTT, 50% glycerol (v/v).

5X RT Buffer

250 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$, 375 mM KCl, 50 mM DTT.

Features

- **Lack RNase H activity:** Weak RNaseH activity. High yield of long length cDNA.
- **Thermal stable:** Stable from 50°C to 60°C. Allow to reduce the effect of secondary structure of RNA during cDNA synthesis.
- **Wide temperature range:** Capable to perform from 37 °C to 60°C.
- **Strong amplification activity:** Gene mutation enhanced the amplification speed and cDNA. Suitable for cDNA library construction.

Recommended Reaction Conditions:

The first-strand cDNA synthesis

1) Add the following reagents to a RNase free PCR tube at room temperature.

Oligo dT_{12-18} (1 μg) or random primer (50-250 ng) or gene-specific primer (2 pmole)	x μL
Total RNA (10 ng - 5 μg) or mRNA (1-500 ng)	x μL
dNTP (10 mM each; Cat. No. 40701)	1 μL
DEPC ddH_2O	to 14 μL

2) Gently mix and incubate 10 min at 70°C then chill on ice for 2-10 min.
3) Centrifuge for few seconds then add the next composition on ice:

5X RT Buffer	4 μL
RNase inhibitor (10 U/μL)	1 μL

4) Gently mix and incubate at 50°C for 2 min (for Oligo dT_{12-18} or gene-specific primer) or at 25°C for 10 min (for random primer).
5) Centrifuge for seconds before adding 1 μL *Pyro*RTase™ (100 U/μL). Incubate at 45 °C to 50°C for 50-70 min.
6) Inactivate *Pyro*RTase™ at 70°C for 10 min.

For research use only.

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