Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

MMLV Reverse Transcriptase Thermal stable

Product Number: RT03

Storage condition

-20°C

Component

MMLV (RNase H-) (200U/uL)
5×RT Buffer (with DTT)

Description

MMLV Reverse Transcriptase, 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. MMLV RT 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. The difference between this to the general MMLV RT is that the capacity to endure the heat is enhanced. It can remain the 100% activity at 50°C, it can also keep more than 80% activity even at 55°C.

Features

Lack RNase H activity: Weak RNase H activity High cDNA yield, can get more full-length cDNA.

Thermal stable: the optimum reaction temperature is 50°C, the highest is 55°C. Can overcome the template RNA secondary structure, and finish the reverse transcriptase experiment smoothly.

Wide temperature range: can reverse transcript from 37-55°C, with more than 80% of the highest activity at 42°C-55°C. customer can choose the reaction temperature freely.

Strong amplification activity: Gene mutation enhanced the binding capacity of the enzyme and RNA. Increased the amplification speed, can obtain the quality cDNA, suitable for cDNA library construction.

Source

Recombination of E. coli containing Moloney murine leukemia virus reverse transcriptase gene from clone of Moloney murine.

Application

The first-strand cDNA synthesis; RT-PCR.

Unit definition

Using Poly (A) as the template and oligo (dT) as the primer, the enzyme required to catalyze the incorporation of 1 nmol of dTTP within 10 minutes at 37°C is defined as one active unit (U).

Storage Buffer

20 mM Tris-HCl (pH7.5), 200 mM NaCl, 0.25 mM EDTA, 0.01% NP-40(v/v), 2.5 mM DTT, 50% glycerol (v/v).

5×Reaction Buffer:

[5×RT Buffer] 250mM Tris-HCl (pH 8.3), 15mM $MgCl_2$, 375 mM KCl, 50mM DTT.

Protocol

The first-strand cDNA synthesis

Add the following reagents to a RNase free PCR tube at room temperature add the MMLV RT last.

Oligo dT12-18 (1μg/μl) or random primer (50-250ng) 1μl

Total RNA (10ng-5μg) or mRNA(1-500ng) xμl

dNTP (10mM each) 1μl

DEPC ddH_2O (14-x)μl

Gently mix and incubate 10 Min at 70°C then chill on ice for 2-10min.

Centrifuge for a few seconds then Put the tube into ice and add the next composition.

5×RT Buffer 4μl

RNasin (40U/μl) 1μl

Gently mix and incubate at 50°C for 2 min (Oligo dT12-18 or sequence especially primer) or at 25°C for 10 min for the random primer.

Centrifuge for a few seconds. Add 1μl MMLV RT (200U/μl) Incubate at 50°C for 50min.

Inactivate at 70°C for 10min then get the cDNA.