MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

ThermoPlus M-MLV Reverse Transcriptase (RNase H-)

Product Number: RT5060

Shipping and Storage

Store at -20℃.

Components

Component	RT5060 200KU
ThermoPlus M-MLV Reverse Transcriptase (RNase H-)	1 ml
5×RT Buffer	1 ml

Description

ThermoPlus M-MLV Reverse Transcriptase(RNase H-),encoded by Moloney Murine Leukemia Virus (M-MLV 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. thermol plus MMLV is a mixture of several temperature-resistant reverse transcriptase enzymes with good temperature adaptability. The optimal temperature range is 50-60 ℃, adapting to a variety of different reaction conditions. It has excellent reverse transcription performance.

Source

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

Concentration

200U/μl

Features

1. Lack RNase H activity: Weak RNaseH activity High cDNA yield, can get more full length cDNA.
2. Thermal stable: the optimum reaction temperature is 50℃,the highest is 60℃. Can overcome the template RNA secondary structure ,and finish the reverse transcriptase experiment smoothly.
3. Wide temperature range: can reverse transcript from 37-60C,with more than 80% of the highest activity at 42℃-55℃ customer can choose the reaction temperature freely.
4. 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.

Application

The first-strand cDNA synthesis; RT-PCR.

Unit definition

One unit of MMLV RT catalyzes the incorporation of 1 nmol of dTTP into acidinsoluble material in 10 minutes at 37℃ using oligo(dT)12-18-primed poly(A)n as a template.

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×RT Buffer:250mM Tris-HCl (pH 8.3), 15mM MgCl2,375 mM KCl,50mM DTT.

For Research Use Only

Recommended Reaction Conditions:

The first-strand cDNA synthesis

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

Component	Volume
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

2. Gently mix and incubate 10 Min at 70℃ then chill on ice for 2-10min.
3. Centrifuge for a few seconds then Put the tube into ice and add the next composition :

Component	Volume
5×RT Buffer	4μl
RNasin (40U/μl)	1μl

4. Gently mix and incubate at 50℃ for 2 Min（Oligo dT12-18 or sequence especially primer）or at 25℃ for 10 min for the random primer.
5. Centrifuge for a few seconds. Add 1μl M-MLV RT（200U/μl）Incubate at 50℃ for 50min.
6. Inactivate at 70℃ for 10min then get the cDNA.