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POLYMERASES & MODIFYING ENZYMES

Product Datasheet

M-MuLV Reverse Transcriptase III

Product No : ME2307
Quantity : 10000u

Lot :
Expiry Date :
Concentration : 200u/µl
Supplied with : 5X Buffer M-MuLV

Store at -20°C

info@vivantechnologies.com

Description:

M-MuLV Reverse Transcriptase III is an upgraded version of M-MuLV Reverse Transcriptase RNase H^- which can perfom higly efficient reverse transcription reactions at 37°C - 55°C. The enzyme is from recombinant E.coli containing the modified *pol* gene of Moloney Murine Leukemia Virus (M-MuLV). The reverse transcription temperature can be increased to 50°C - 55°C, avoid the inhibition of cDNA synthesis by RNA complex secondaey structure, and can effectively synthesize high-quality cDNA. It can generate cDNA from 100bp to >12 kb. M-MuLV Reverse Transcriptase III has superior continuous synthesis ability and super strong impurity tolerance.

Unit Definition:

1u is defined as the amount of the enzyme that is required to incorporate 1nmol of dTTP into an acid-insoluble material in 10 minutes at 37°C using poly(rA)•oligo (dT).

Storage Buffer:

20mM Tris-HCl, 100mM NaCl, 0.1 mM EDTA, 1mM DTT, 50% (v/v) glycerol.

Reaction Buffer:

5X Buffer M-MuLV

250mM Tris-HCl, 375mM KCl, 15mM $MgCl_2$ and 50mM DTT.

Thermal Inactivation: 85°C for 5 seconds

Application:

- First strand cDNA synthesis.
- DNA labeling.
- RNA analysis by primer extension.

Quality Control:

Purified free of detectable levels of RNase, endonuclease and exonuclease activities.

Product Use Limitation
This product is for research purposes and *in vitro* use only.

Recommended Protocol for first strand cDNA synthesis

1. Mix the reagents well and centrifuge the tubes briefly before pipetting.
2. Prepare the RNA-primer mixture as below in a 2.0ml microcentrifuge tube.

A. RNA-primer mixture

Indicator	Suggested Concentration
Template: Total RNA or Poly A^+ mRNA	10 pg - 5 µg 10 pg - 500 ng
Primer: Oligo d(T) 12-18 or Random Hexamers or Gene Specific Primer (GSP)	200 - 500 ng 100 µM Depend on application
10mM dNTPs Mix	1 µL
Nuclease-free water	Top up to 10 µL

3. Incubate the mixture at 65 °C for 5 minutes and then chill on ice immediately for 2 minutes.
   *The denaturation step helps to open the secondary structures to improve the first strand cDNA yield. For cDNA fragment longer than 3kb, do not ignore the denaturation step.
4. Briefly spin down the mixture.
5. Prepare the following cDNA synthesis mixture in the order indicated:

B. cDNA synthesis mixture

Component	Amount/Volume
5X Buffer M-MuLV III	4 µL
M-MuLV Reverse Transcriptase III	100 - 200U
Nuclease-free water	Top up to 10 µL

6. Add 10µl of the cDNA synthesis mixture into each RNA-primer mixture. Mix gently and centrifuge briefly.

C. Cycling program

Temperature:	Time
25°C	5 minutes
55°C	15 - 45 minutes
85 °C	5 seconds

[a] This step is required only when using the Random Hexamers. Omit this step using Oligo(dT) or GSP.

Recommended Protocol for One Step Reverse Transcription - PCR

1. Mix the reagents well and centrifuge the tubes briefly before pipetting.
2. Add the following component in a 2.0ml microcentrufuge tube on ice.

A. For 20µL reaction volume:

Reagent:	Final Concentration	Amount/Volume
10X PCR Buffer	1X	2.0 µL
50mM $MgCl_2$	1.5 - 3mM	0.6 µL
10mM dNTPs mix	0.4 mM	0.2 µL
5U Taq / HotStart Taq	1 - 2U	0.2 - 0.4 µL
M-MuLV Reverse Transcriptase III	100 - 200U	0.5 - 1.0 µL
Primer (Fwd / Rev)	0.1 - 1 µM each	
Total RNA Poly A^+ RNA	10 pg - 5 µg 10 pg - 500 ng	
RNase free ddH_2O	Adjust final volume to 20 µL	

* Higher reaction volume may be achieved provided that the same final concentration of each reaction component is maintained. The number of tests will be reduced if the reaction volume more than 20µL.

B. Cycling program

Step	Cycles	Temp	Time
Reverse Transcription	1	55°C	15 minutes
Initial Denaturarion	1	94°C	2 - 8 minutes
Denaturation	40	94°C	10 - 30 seconds
Annealing		50 - 70°C	30 - 60 seconds
Final Extension		72°C	1 minutes
Data Collection*			

* For real-time PCR, fluorogenic data should be collected during step.
** This protocol may change depending on the template RNA and primers used

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