Tinzyme Co., Limited

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Reverse Transcriptase III

Product Number: RT09

Shipping and Storage

Store at -30-15 ℃ and transport at ≤ 0 ℃.

Components

Component	RT09
Reverse Transcriptase III (200 U/μl)	50μl
5×Reverse Transcriptase III Buffer	250μl

Description

Reverse Transcriptase III is a novel reverse transcriptase obtained through molecular evolution technology screening in E.coli expression system in vitro. Compared with the original reverse transcriptase, its cDNA synthesis speed is fast, and its thermal stability is greatly improved. It can withstand reaction temperatures up to 60℃ and is suitable for reverse transcription of RNA templates with complex secondary structures. At the same time, the enzyme enhances its affinity with templates, making it highly suitable for reverse transcription of small amounts of templates and low copy genes. The ability of Reverse Transcriptase III to synthesize full-length cDNA has also been improved, with the ability to expand and grow up to 19.8 kb of cDNA.

Application

Construction of a full-length cDNA library; End point PCR; Real time quantitative PCR, etc.

Unit definition

Using Poly (rA)·Oligo (dT) as a template/primer, the enzyme amount required to add 1 nmol of dTTP as an acid insoluble substance is defined as 1 active unit (U) at 37 ℃ and 10 minutes.

Note

1. Please keep the experimental area clean;
2. Wear clean gloves and masks during operation;
3. The consumables used in the experiment, such as centrifuge tubes and gun heads, must ensure RNase free.

Protocol

Steps for the synthesis of the first strand cDNA

1. RNA denaturation (This step is an optional step, as RNA denaturation helps to open the secondary structure and can greatly increase the production of the first stranded cDNA.)

Component	Volume
RNase-free H_2O	to 13μL
Oligo (dT)18 (50μM) or Random Primers (50μM) or Gene Specific Primers (2μM)	1μL
Template RNA	Total RNA: 10 pg -5μg or mRNA:10 pg-500 ng

Heat at 65 ℃ for 5 minutes and quickly place on ice to cool for 2 minutes. Collect the reaction solution briefly by centrifugation, add the reverse transcription reaction solution in the table below, and gently blow and mix with a pipette.

2. Preparation of Reverse Transcription Reaction System (20μL system)

Component	Volume

The reaction solution from the previous step	13μL
5×Reverse Transcriptase III Buffer	4μL
dNTP Mix (10 mM)	1μL
Reverse Transcriptase III (200 U/μl)	1μL
RNase inhibitor (40 U/μL)	1μL

3. Reverse transcription program settings

Temperature	Time
25℃	5 min
55℃	15-30min
85℃	5 min

Note: 1) When using Random Primers, incubate at 25℃ for 5 minutes; If using Oligo (dT) 18 or Gene Specific Primers, this step can be omitted;

2)Reverse transcription temperature: It is recommended to use 55℃; For high GC content templates or complex templates, the reverse transcription temperature can be increased to 60℃;

3)Extending the reverse transcription time to 45-60 minutes can help increase yield;

4)Heat at 85℃ for 5 minutes to inactivate reverse transcriptase.

※**Reverse transcripts can be immediately used for subsequent PCR or qPCR reactions, and can also be stored for a short period of time at -20℃. If long-term storage is required, it is recommended to separate and store at -80℃ to avoid repeated freeze-thaw cycles.**