Tinzyme Co., Limited

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X Reverse Transcriptase

Product Number: RT10

Shipping and Storage

Storage at -25~-15℃ and transportation≤ 0℃. Avoid repeated freezing and thawing, and valid for 18 months.

Component

Component	RT10
X Reverse Transcriptase (Glycerol-free)(15U/μL)	0.1mL
10 × X RT Buffer	1.5mL
$MgSO_4$ (10 mM)	1.5mL

Description

X Reverse Transcriptase is an aptamer-modified and RNA-template-dependent DNA polymerase that inhibits its reverse transcription activity below 40°C. X Reverse Transcriptase lacks 3' → 5' exonuclease activity and has RNase H activity. The enzyme can use RNA as a template to synthesize a complementary DNA strand, which can be applied to first-strand cDNA synthesis. Because of its high activity between 50-65°C, it is particularly suitable for RT-LAMP (Loop-Mediated Isothermal Amplification). Since the polymerase activity of X Reverse Transcriptase is inhibited below 40°C, the non-specific amplification caused by mismatching of primers during the preparation of the reaction system can be greatly reduced, solving the problems of primer-dimer formation before the reaction, improving the consistency and specificity of amplification. X Reverse Transcriptase supports high-throughput applications and operation at room temperature. X Reverse Transcriptase (Glycerol free) can be used to prepare lyophilized preparations, lyophilizable RT-LAMP reagents, etc.

Application

1. cDNA synthesis;
2. Combined with Bst DNA;
3. RT-LAMP isothermal amplification can be performed to detect target RNA.

Unit definition

One unit incorporates 1nmol of dTTP into acid-precipitable material in 20 minutes at 50°C using poly(A)•oligo(dT) as template-primer.

Quality control

1. Endonuclease Activity: Incubation of 15 U of enzyme with 1μg λ DNA for 16 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis.
2. Exonuclease Activity: Incubation of 15 U of enzyme with 1μg λ-Hind III digest DNA for 4 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis.
3. Nickase Activity: Incubation of 15 U of enzyme with 1μg pBR322 for 4 hours at 37°C resulted in consistent with negative control of the DNA as determined by gel electrophoresis.
4. RNase Activity: Incubation of 15 U of enzyme with 0.48μg MS2 RNA for 4 hours at 37°C resulted in consistent with negative control of the RNA as determined by gel electrophoresis.

Protocol

1. **cDNA Synthesis Protocol**

Component	Volume
Template RNA	Variable
Oligo(dT)18~25(50μM) or Random Primer Mix(60μM)	2μL
dNTP Mix (10 mM each)	1μL
RNase Inhibitor(40 U/μL)	0.5μL
X Reverse Transcriptase (Glycerol-free)(15U/μL)	0.5μL
10 × X RT Buffer	2μL
Nuclease-free Water	Up to 20μL

Note: The recommended dosage of total RNA is 1ng~1μg; The recommended dosage of mRNA is 50ng~100ng.

2. Reaction procedure

Temperature	Time
25℃	5min
55℃	10min
80℃	10min

Note: If Random Primer Mix is used, an incubation step at 25°C.

The incubation time at 55°C can be adjusted for 10~30 min. Longer reverse transcription time helps to obtain longer cDNA (>5 kb).

3. **RT-LAMP Protocol**

Component	Volume	Fin. Con
Template RNA	Variable	≥10copies
dNTP Mix (10mM)	3.5μL	1.5mM
FIP/BIP Primers (25×)	1μL	1.6μM
F3/B3 Primers (25×)	1μL	0.2μM
LoopF/LoopB Primers (25×)	1μL	0.4μM
RNase Inhibitor(40 U/μL)	0.5μL	20U/Rxn
X Reverse Transcriptase（Glycerol-free)(15U/μL)	0.5μL	7.5U
Bst DNA Polymerase (8 U/μL)	1μL	8U
$MgSO_4$ (100mM)	1μL	6mM (Total8mM）
10 × X RT Buffer	2.5μL	1× (Contain 2mM Mg^{2+})
Nuclease-free Water	Up to25μL	/

Mix by vortexing and centrifuge briefly, then keep at 65°C for 1 hour.

Note: The two buffers are interoperable and have the same composition.

Note

1. This product may appear white precipitate after stored at -20°C. It is a normal observation. Please keep it in ice for 10 min to make the precipitate get dissolved and mix well before use.
2. The cDNA product can be stored at -20°C or -80°C or used immediately for PCR reaction.
3. To prevent RNase contamination, pleasekeep the working area clean, and wear cleaned gloves and masks during operation. The centrifugal tube, suction and other consumables used in the experiment should be guaranteed to be RNase-free.