RTL Reverse Transcriptase (Glycerol-free)

rp216167

Description:

RTL reverse transcriptase 2.0 is an RNA template-dependent DNA polymerase that lacks the 3' → 5' exonuclease activity and has RNase H activity. This enzyme can use RNA as a template to synthesize a complementary strand of DNA, which can be applied to first-strand cDNA synthesis, especially for RT-LAMP (loop-mediated isothermal amplification). Compared with RTL reverse transcriptase 1.0, the sensitivity is significantly improved, the thermal stability is stronger, and the reaction at 65°C is more stable. RTL reverse transcriptase 2.0 (glycerol free) can be used to prepare freeze-dried preparations, freeze-dried RT-LAMP reagents, etc

Storage and transportation

Storage at -25~-15°C and transportation ＜0°C.

Unit definition

One unit incorporates 1 nmol of dTTP into acid-precipitable material in 20 minutes at 50°C using poly(A)•oligo(dT)25 as template-primer.

Composition of product

RTL Reverse Transcriptase 2.0 (Glycerol-free)（15U/μL）	0.1mL
10 × HH RTL Buffer	1.5mL
MgSO4(100 mM)	1.5mL

Quality Control Assays

Residual Activity of Endonuclease: A 50 μL reaction containing 1 μg of λDNA and 15 units of RTL2.0 incubated for 16 hours at 37°C shows same pattern as negative control by gel electrophoresis.

Residual Activity of Exonuclease: A 50 μL reaction containing 1 μg of Hind III digested λ DNA and 15 units of RTL2.0 incubated for 4 hours at 37°C shows same pattern as negative control by gel electrophoresis.

Residual Activity of Nickase: A 50 μL reaction containing1 μg of supercoiled pBR322 and 15 units of RTL2.0 incubated for 4 hours at 37°C shows same pattern as negative control by gel electrophoresis.

Residual Activity of RNase: A 10 μL reaction containing 0.48 μg of MS2 RNA and 15 units of RTL2.0 incubated for 4 hours at 37°C shows same pattern as negative control by gel electrophoresis.

E.coli gDNA: Measured with E.coli specific HCD detection kits , 15 units of RTL2.0 contains less than 1 E. coli genome.

Reaction setup

Component	Volume
Template RNAa	optional
Oligo(dT)18~25（50μM）or Random Primer Mix（60 μM）	2 μL
dNTP Mix (10 mM each)	1 μL
RNase Inhibitor（40 U/μL）	0.5 μL
RTL Reverse Transcriptase 2.0 (15 U/μL)	0.5 μL
10 x HH RTL Buffer	2 μL
Nuclease-free Water	Up to 20 μL

Note: The recommended dosage of Total RNA is 1ng~1μg
The recommended dosage of mRNA was
50ng~100ng

Thermocycling Conditions for a routine reaction：

Temperature（°C）	Time
25 °Cⓐ	5 min
55 °C	10 minⓑ
80 °C	10 min

ⓐIf Random Primer Mix is used, an incubation step at 25°C.
ⓑIf target primer mix is used, an incubation step at 55°C for 10~30 min.

RT-LAMP Protocol

Component	Volume	Fin. Con
Template RNA	optional	⩾10copies
dNTP Mix (10 mM)	3.5 μl	1.4 mM
FIP/BIP Primers (25×)	1 μL	1.6 μM
F3/B3 Primers (25×)	1 μL	0.2 μM
LoopF/LoopB Primers (25×)	1 μl	0.4 μM
RNase Inhibitor（40 U/μL）	0.5 μL	20U/Reaction
RTL Reverse Transcriptase 2.0 (15 U/μL)	0.5 μL	7.5 U/Reaction
Bst V2 DNA Polymerase(8 U/μL)	1 μL	8 U/Reaction
MgSO4 (100 mM)	1.5 μL	6 mM (Total 8 mM)
10 x HH RTL Buffer(or 10 x HH Bst V2 Buffer*)	2.5 μL	1 ×(2 mM Mg2+)
Nuclease-free Water	Up to 25 μL	—

Mix by vortexing and centrifuge briefly to collect. Constant temperature incubation at 65°C for 1 hour.

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

Precaution

1)This product will form a white solid when stored at -20 °C. Take it out from -20°C and put it on ice for about 10 minutes. After melting, it can be used by shaking and mixing.

2)The cDNA product could be stored at -20°C or -80°C or used immediately for PCR reaction.

3)To prevent RNase contamination, please keep the experimental area clean, and wear clean gloves and masks during operation.