RTL Reverse Transcriptase（Glycerol free, high density）

Cat.No.:HYB213

Product Description

RTL reverse transcriptase 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. RTL reverse transcriptase (glycerol free) can be used to can be used to prepare freeze-dried preparations, freeze-dried RT-LAMP reagents, etc.

Components

Components	1500U	15000U	150000U
RTL Reverse Transcriptase (Glycerol-free,high density) (75U/μL)	0.02mL	0.2 mL	2 mL
10 × HH RTL Buffer	1.5mL	4×1.5mL	5×10mL
$MgSO_4$（100 mM）	1.5mL	2×1.5mL	3×10mL

Storage

Store at -25～-15 °C, valid for 18 months.

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.

Quality control

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

2. **Residual Activity of Exonuclease:** A 50μL reaction containing 1 μg of Hind III digested λ DNA and 15 units of RTL incubated for 4 hours at 37°C shows same pattern as negative control by gel electrophoresis.
3. **Residual Activity of Nickase:** A 50 μL reaction containing1 μg of supercoiled pBR322 and 15 units of RTL incubated for 4 hours at 37℃ shows same pattern as negative control by gel electrophoresis.
4. **Residual Activity of RNase:** A 10 μL reaction containing 0.48 μg of MS2 RNA and 15 units of RTL incubated for 4 hours at 37℃ shows same pattern as negative control by gel electrophoresis.
5. ***E.coli* gDNA:** Measured with *E.coli* specific HCD detection kits，15 units of RTL contains less than 1 *E. coli* genome.

Reverse transcription reaction system

Component	Volume
Template RNA	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 (Glycerol-free,high density) (75U/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.

Reaction program

Temperature	Time
25 ℃*	5 min
55 ℃	10 min**
80 ℃	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 reaction system

Component	Volume	Final concentration
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/Rxn
RTL Reverse Transcriptase (Glycerol-free,high density) (75U/μL)	0.1 μL	7.5 U
Bst 2.0 DNA Polymerase (8 U/μL)	1 μL	8 U
$MgSO_4$ (100 mM)	1.5 μL	6 mM (Total 8mM)
10 x HH RTL Buffer / 10 x HH Bst 2.0 Buffer	2.5 μL	1 ×(2 mM Mg^{2+})
Nuclease-free Water	Up to 25 μL	-

Mix by vertexing and centrifuge briefly to collect. Constant temperature incubation at 60°C-65°C for 1 hour.

Notes

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