Warm Start RTL Reverse Transcriptase（Glycerol free）

Cat.No.:HYB214

Product Description

Warm Start RTL reverse transcriptase is an aptamer-modified RNA-dependent DNA polymerase, whose reverse transcription activity can be inhibited below 40°C. RTL lacks 3' → 5' exonuclease activity and has RNase H activity. It can use RNA as a template to synthesize a complementary DNA chain and can be applied to first-chain cDNA synthesis. Due to its high activity between 50-65°C, it is particularly suitable for RT-LAMP (loop-mediated isothermal amplification). Since the polymerase activity of WS RTL is inhibited below 40°C, it can greatly reduce the nonspecific amplification caused by primer mispairing during the preparation of the reaction system, solve the problem of primer dimers that are easy to generate before the machine reaction, improve the consistency and specificity of amplification, and support high-throughput applications and room temperature operation. WS RTL (Glycerol free) can be used to prepare lyophilized preparations, lyophilizable RT-LAMP reagents, etc.

Components

Components	1500 U	15000 U	150000 U
Warm Start RTL Reverse Transcriptase（Glycerol free）（15U/μL）	0.1mL	1mL	10mL
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 37ºC shows same pattern as negative control by gel electrophoresis.
2. **Residual Activity of Exonuclease:** A 50μL reaction containing 1 μg of Hind III digested λDNA 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 37ºC 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 37ºC 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
Warm Start RTL Reverse Transcriptase (Glycerol free) (15U/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 ºC*	5 min
55 ºC	10 min**
80 ºC	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
Warm Start RTL Reverse Transcriptase (Glycerol free) (15U/μL)	0.5 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