RTv Reverse Transcriptase

RV101

Version 23.1

Product Description

RTv Reverse Transcriptase is obtained from the M-MLV Reverse Transcriptase by directed genetic engineering, which has excellent reverse transcription efficiency, specificity, sensitivity and thermal stability. It is applicable for Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP). With the new generation of hot start technology, the reaction system can be prepared at room temperature. Moreover, the enzyme activity is inhibited at temperatures below 45°C, thus improving specificity.

Components

Components	RV101-01 (1,500 U)
RTv Reverse Transcriptase (15 U/μl)	100 μl
10 × IsothermalAmp Buffer	1 ml
$MgSO_4$ (100 mM)	1 ml

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP).

Source

A recombinant *E. coli* strain carrying modified M-MLV (H-) reverse transcriptase gene.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 20 min at 50°C, with Poly(rA)·Oligo (dT) as the template/primer.

Self-prepared Materials

Reagents: Bst DNA Polymerase Large Fragment, RNase inhibitor, dNTP Mix (10 mM each), FIP/BIP Primers，F3/B3 Primers, LoopF/LoopB Primers, RNase-free ddH_2O。

Instruments: qPCR instrument, PCR instrument or water bath.

Notes

For research use only. Not for use in diagnostic procedures.

- Prevent RNase contamination

  Please keep the experiment area clean; Wear disposable gloves and masks; Use RNase-free consumables such as centrifuge tubes and pipette tips.

Experiment Process

Take RT- LAMP as an example:

1. Thaw components on ice. Vortex for 10 sec to mix thoroughly before use, then centrifuge briefly to the bottom of the tube.

2. Follow the table below to prepare the reaction system. The template should be added in the last step.

Components	Volume	Final Concentration	
10 × IsothermalAmp Buffer	2.5 μl	1 ×	
$MgSO_4$ (100 mM)	1.5 μl	6 mM (total 8 mM)	
dNTP Mix (10 mM each)	3.5 μl	1.4 mM each	
FIP/BIP Primers (100 μM)	0.4 μl each	1.6 μM each	
F3/B3 Primers (100 μM)	0.05 μl each	0.2 μM each	
LoopF/LoopB Primers (100 μM)	0.2 μl each	0.8 μM each	
RNase Inhibitor (40 U/μl)	0.5 μl	0.8 U/μl	
RTv Reverse Transcriptase (15 U/μl)	0.5 μl	0.3 U/μl	
Bst DNA Polymerase Large Fragment (8 U/μl)	1.0 μl	0.32 U/μl	
RNA Template*	1.0 - 5.0 μl		
RNase-free ddH_2O	up to 25 μl		

* It is recommended to add the template last to ensure the reliability of the results.

▲ The concentration of Mg^{2+} can be adjusted between 6 - 10 mM.

▲ If the experiments requires an anti-contamination system, it is recommended that add dUTP (Vazyme #P033) to a final concentration of 1.4 mM, and UDG enzyme to a final concentration of 0.04 U/μl.

▲ If the amount of primers is small, it is recommended to premix the primers first.

▲ It is recommended to prepare reagents and templates in different areas to avoid contamination.

3. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.

4. Add template DNA. The final volume of the reaction system should be 25 μl.

5. Vortex to mix thoroughly, then centrifuge briefly to the bottom of the tube.

6. Incubate at 60 ~ 65°C for 30 - 60 min.

FAQ & Troubleshooting

◇ How to design and screen primers for loop-mediated isothermal amplification?

Please refer to http://primerexplorer.jp/e/ for primer design. Version 5 is recommended.

Log in to http://primerexplorer.jp/lampv5e/index.html to download the manual.

For preliminary screening, please refer to the manual. The optimal primer need to be verified by experiments.

◇ How to detect the amplification product?

Both dye-based method and probe-based method can be used to detect amplification products.