EZassay

RT-RPA Reverse Transcriptase Instructions

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Catalog Code: RT-RP-2000
RT-RP-10000

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Product Information	
Product name	RT-RPA Reverse Transcriptase
Expression system	Escherichia coli
Quality	Recombinant protein
Form	Liquid

Product Introduction

Reverse Transcriptase (for RPA) is an engineered M-MLV reverse transcriptase specifically optimized for RT-RPA applications.
Compared with conventional M-MLV reverse transcriptase, it exhibits enhanced tolerance to common amplification inhibitors, enabling reliable cDNA synthesis from challenging RNA samples. The enzyme maintains high activity under the mild reaction temperatures (37–45°C) required for RPA, making it particularly suitable for rapid isothermal RNA detection.
It supports rapid reverse transcription with high sensitivity and robust performance across a wide range of RNA templates.

Materials supplied

Cat:	RT-RP-2000	RT-RP-10000
RT-RPA Reverse Transcriptase (200U/μL)	2000U	10000U

Storage

-20°C. Aliquot after receiving. Avoid repeated freeze-thaw.

Unit definition

One unit is defined as the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C.

Features

- Engineered M-MLV reverse transcriptase optimized for RT-RPA
- Excellent activity at 37–45°C
- High inhibitor tolerance
- Robust performance with complex RNA samples
- Compatible with one-step RT-RPA workflows
- High cDNA synthesis efficiency
- Suitable for rapid molecular diagnostics

Example of application in RT-RPA

1. Set incubator working temperature at 42 °C. (Optimization range 37~45°C) (Turn off the lid heating function or set to 42 °C if PCR thermocycler is used.)
2. For each reaction, add the reagents as described in the table below. It is recommended to set up the reactions on ice. To minimize pipetting variation, prepare a master mix according to the total number of reactions.

Component	RT-RPA	Working concentration
RPA reaction buffer (2X)	10 µL	1X
dNTP Mix (10 mM each)	0.4µL	0.2 mM (each)
Forward primer*	Varies	0.3 µM
Reverse primer	Varies	0.3 µM
T4 UvsX protein	0.5 µL	0.03 mg/mL
T4 UvsY protein	0.5 µL	0.03 mg/mL
T4 Gene 32 protein	0.5 µL	0.4 mg/mL
Bsu DNA Polymerase（large fragment）(6 U/µL)**	0.5 µL	0.15 U/µL
RT-RPA Reverse Transcriptase (200U/µL)	0.2~0.6 µL	2~6 U/µL
RNA template	Varies	-

Murine RNase Inhibitor	0.8 μL	1.6 U/μL
Mg(OAc)2（280 mM）***	1μL	14 mM
Nuclease-free water	Up to 25μl	-

* A final primer concentration of 0.3 μM per primer is recommended for singleplex (RT-)RPA reactions. For multiplex (RT-)RPA reactions, reduce the concentration of each primer to 0.1 μM. If necessary, primer concentrations may be further optimized within the range of 0.1–0.3 μM.
** The final concentration of Bsu Polymerase in the reaction may range from 0.015 to 0.15 U/μL. In some cases, a lower polymerase concentration may improve the specificity and sensitivity of target amplification.
*** $Mg(OAc)_2$ should be added last and individually to each reaction, as it initiates the RPA reaction.

3. Mix thoroughly by gently flicking the tube, then briefly spin down. Repeat this process 3 times.
4. Incubate at 42°C for 20–40 minutes to generate sufficient amplicons.
5. It is recommended to purify the amplification products before agarose gel electrophoresis to better visualize the amplified DNA products.

Comparison of Amplification Performance in the RT-RPA System

Component	RT-RPA Reverse Transcriptase	M-MLV (H-)
Time to Threshold (Tt)	5 min	8 min
Fluorescence intensity	19470 RFU	10860 RFU

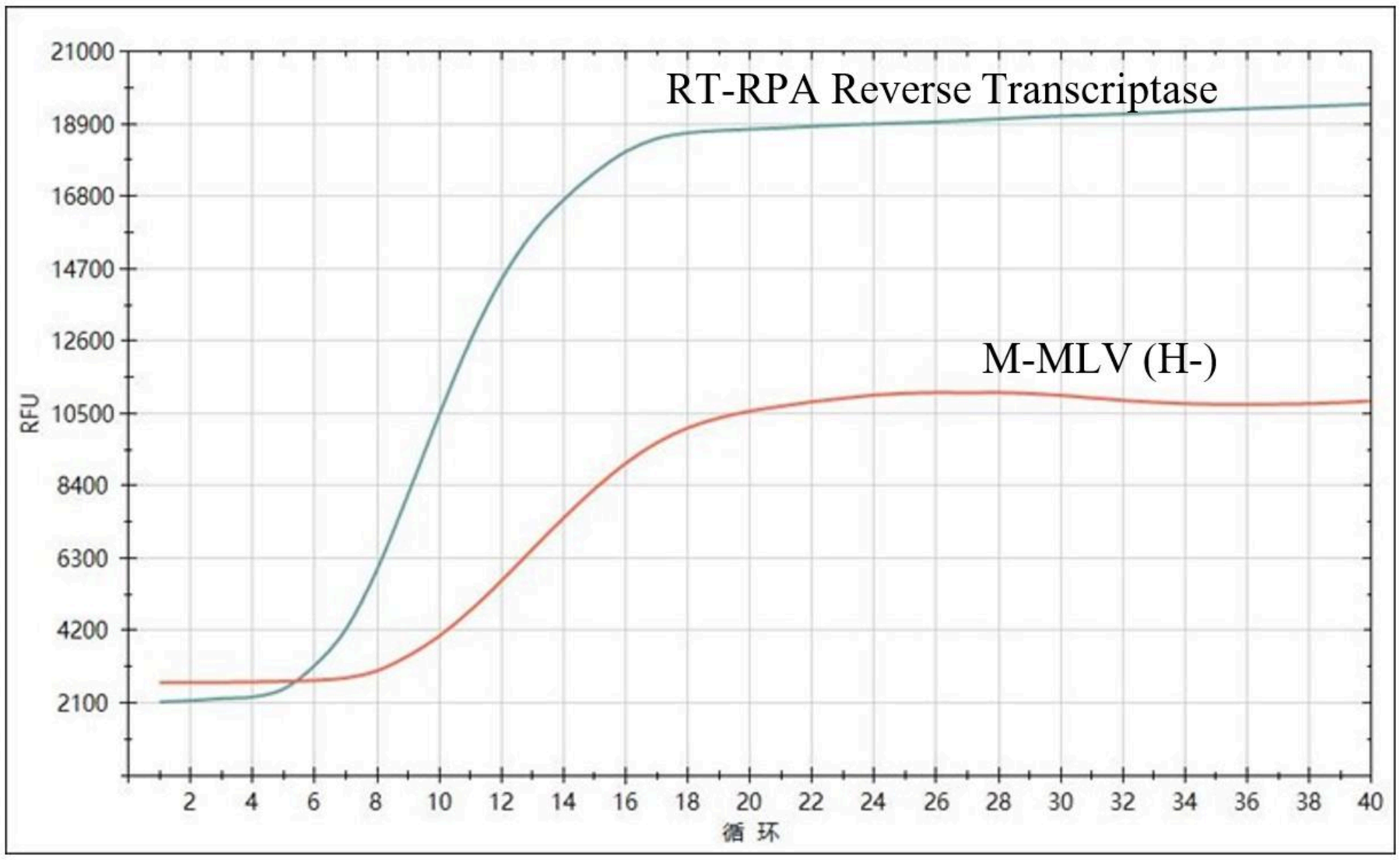

Figure 1. Comparison of the Performance of RT-RPA-Specific Reverse Transcriptase and M-MLV (H-) in RT-RPA