EZassay

Reverse Transcriptase (for RPA) Instructions

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

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Product Information	
Product name	Reverse Transcriptase (for RPA)
Expression system	Escherichia coli
Quality	Recombinant protein
Form	Liquid

Product Introduction

Reverse Transcriptase is an RNA-dependent DNA polymerase that synthesizes complementary DNA (cDNA) from single-stranded RNA, single-stranded DNA, or RNA:DNA hybrid templates. It can be used for cDNA synthesis from RNA templates. Reverse Transcriptase (for RPA) is an engineered mutant of M-MLV reverse transcriptase, specifically evolved and optimized for RT-RPA applications.

Storage

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

Materials supplied

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

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.

Example of application in RT-RPA

1. Set incubator working temperature at 42 °C. (Optimization range 37~42°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	Volume	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
Reverse Transcriptase (for RPA) (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	-

*0.3 µM is recommended primer concentration for single-plex (RT-)RPA reactions. For multiplex (RT-)RPA, reduce primer concentration to 0.1 µM each. If required, primer concentration may be optimized in the 0.1–0.3 µM range.
*Bsu Polymerase concentration in the reaction can range between 0.015–0.15 U/µL. In some cases, lower polymerase concentration can help to achieve higher specificity and sensitivity of target amplicon.
*Mg(OAc)2 has to be added last and separately to each sample (Mg(OAc)2 initiates the reaction)

3. Mix thoroughly by gently flicking the tube, then briefly spin down. Repeat this process 3 times.
4. Incubate at 39–42°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.

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