

Reverse Transcriptase

(10000 units)



MO5432

Concentration: 200u/μl



Wet ice



Store at: -20°C

Components

Contents	Quantity/ Volume	Store Temperature
Reverse Transcriptase (200u/μl)	50μl	-20°C
5X Reaction Buffer	400μl	-20°C

Description

Reverse Transcriptase is a recombinant Enzyme with reduced RNase H activity and increased thermostability. The enzyme is active up to 55°C. It provides higher specificity, higher yield and more full-length cDNA products.

Features

- Increased thermostability for more full-length cDNA products.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- cDNA up to 15 kb.

(MRT-040-01/00) (1)



Unit Definition: One activity unit (U) refers to the amount of reverse transcriptase when catalyzes the incorporation of 1nmol of dTTP into materials in 10min at 37°C using oligo (dT) primed poly (A) as a template.

5X Reaction Buffer

300mM KCl, 20mM MgCl₂; 20mM DTT; 125mM Tris-HCl (pH=8.3).

Storage Buffer

50mM Tris-HCl (pH=7.5), 150mM NaCl, 1mM DTT, 50% glycerol.

First Strand cDNA synthesis (20μl reaction volume)

1. Add components according to the below table:

Components	Volume
Total RNA/mRNA	50ng-5μg / 5-500ng
Oligo(dT)18 (0.5μg/μl)	1μl
Or Random Primer (0.1μg/μl)	1μl
Or GSP (Gene Specific Primer)	2pmol
dNTP Mix, 10 mM each	1μl
5 X RT Buffer	4μl
Ribonuclease Inhibitor (40 units/μl)	0.5μl
Reverse Transcriptase	1μl
RNase free H ₂ O to final volume	20μl

Optional (if RNA template is GC-rich or is known to contain secondary structures).

Suggest to mix RNA/ Primer/ RNase free H₂O gently and briefly centrifuge, incubate at 65°C for 5min, chill on ice and briefly centrifuge, then place the tube on ice. Add other components and continue.

(2)

2. Mix well gently

If Oligo(dT)₁₈ or gene specific primer (GSP) are used, incubate at 50°C for 30-50min.

If Random Primer is used, incubate 10min at 25°C followed by 30-50min at 55°C.

3. Terminate the reaction by heating at 70°C for 15min.

The reverse transcription reaction product can be directly used in PCR or stored at -20°C.

RT-PCR

Use 2-4μl of the reaction mix to perform PCR in 50μl volume.

PCR mixture set up (for 50μl reaction volume)

Components	Volume	Final Concentration
cDNA Template	2-4μl	as required
Forward Primer (10μM)	1μl	0.2μM each
Reverse Primer (10μM)	1μl	0.2μM each
10X Taq Buffer (contains Mg ²⁺)	5μl	1X
2.5mM dNTPs	4μl	0.2mM
Taq DNA Polymerase	0.5μl	2.5 units
ddH ₂ O to final volume	50μl	Not applicable

PCR Condition





94°C	2-5 min	1 Cycle
94°C	30 sec	30-40 Cycles
Variable	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	1 Cycle

(3)

Quality Control

Purified free of detectable levels of RNase, endonuclease and exonuclease activities.

Signs

Signs	Definitions	Signs	Definitions
	For Research Use Only		Name and address of the manufacturer of the product
	Product shipping conditions		Product technical code

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(4)