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## Product Manual

<b>Product Name</b>	RTX Reverse Transcriptase
<b>Source</b>	Recombinant expression in Escherichia coli
<b>Catalog Number</b>	CSB-DEM046
<b>Physical Form</b>	Liquid
<b>Enzyme Activity</b>	200 U/ $\mu$ L
<b>Storage Conditions</b>	-20 $\pm$ 5 $^{\circ}$ C
<b>Storage Buffer</b>	20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% NP-40, 50% glycerol
<b>Activity Definition</b>	The amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble material in a 50 $\mu$ L reaction system using Poly(rA) $\cdot$ Oligo d(T)15 as a template at 37 $^{\circ}$ C for 10 minutes is defined as 1 unit (U).
<b>Quality Control</b>	No detectable nuclease, DNase, and RNase activities. PCR detection confirms the absence of residual host genomic DNA.
<b>Shelf Life</b>	24 months

### Product Description

RTX Reverse Transcriptase is a newly engineered reverse transcriptase enzyme through genetic modification. RTX possesses higher thermal stability and salt tolerance, making it suitable for reverse transcription of RNA templates with secondary structures. It exhibits optimal activity at temperatures between 60-70 $^{\circ}$ C and has both DNA and RNA polymerase activities, allowing for cDNA synthesis or direct one-step RT-PCR and RT-qPCR. Inactivation can be achieved by heating at 95 $^{\circ}$ C for 3 minutes.

## Product components

Component No.	Component Name	Specifications		
		0.2mL	1mL	2mL
1	10×RTX Buffer			
2	RTX Reverse Transcriptase	20000U	100000U	200000U

## Operating instructions

### cDNA synthesis

Add and mix each component according to the table below, incubate at 60 ° C for 0.5h.

Then, heat at 95 ° C for 3 minutes to inactivate the enzyme, and store the reverse transcript at -20 ° C

Composition	Addition amount
ddH <sub>2</sub> O	Up to 20 μL
10×RTX Buffer	2μL
RNA template*	50 pg -1 μg*
50 μM d(T)23VN or 60 μM random primers	2 μL
10 mM dNTPs	1 μL
RNase Inhibitor (40 U/μL)	0.2 μL
RTX Reverse Transcriptase(200 U/μL)	1 μL

**Note:** 1 ng-1 µg total RNA template or 50 pg-100 ng Poly(A)-RNA. Inactivation at 95°C for 3 minutes. For downstream PCR applications, the volume of reverse transcription product added should not exceed 1/10 of the total PCR reaction volume.

### One-Step RT-qPCR Reaction

Generally, the recommended concentration of RTX Reverse Transcriptase for a reaction is between 20-30u/30 µ L. The amplification conditions are as follows:

#### Recommended PCR reaction program

Temperature	Time	Cycles
60-70°C	5-10 min	1
95°C	3 min	1
95°C	10 s	25-35
45-68°C	20 s	
68°C	1 kb/min	
72°C	5 min	1

**Note:**

Annealing temperature and time: Annealing temperature should be adjusted based on the primer's T<sub>m</sub> value, generally set to 3-5°C below the primer T<sub>m</sub> value. Recommended annealing time is 20 sec, adjustable within 10-30 sec