Research use only

Thermophilic Reverse Transcriptase

Product Name: Thermophilic Reverse Transcriptase:
Cat. #: W140 (200 unit / reaction)

Applications

- The reverse transcriptase is a thermophilic type A polymerase (US patent pending) with optimal temperatures at 60-62°C, and can be heat-inactivated at ≥90°C.
- The enzyme efficiently synthesizes a complementary DNA strand initiating from a gene-specific primer, *one unit per 20 µL of reaction.*
- The enzyme can detect single digit copies of target RNA depending on assay design and optimization.
- It is particularly suitable to one-step real-time qualitative and quantitative RT-PCR.

Enzyme properties

Reverse transcriptase activity	Yes
5'-3' DNA polymerase activity	Yes
5'-3' exonuclease activity	Yes
3'-5' exonuxlease activity	No
Incorporation of modified nucleotides	Yes, such as dUTP, fluorescence dye-labeled dNTPs
Heat-Inactivated	≥90°C for 2 min
DNase or RNase activity	Not detectable

Optimal parameters

Optimal RTase amount	1-2 U per 20 ul reaction
Optimal temperature	60-62°C
Optimal incubation time	10 min
Mg_2 concentration	1.5-3 mM
dNTP concentration	Each 40-200 uM
Product size	Preferred ≤150 bp

Unit definition

One unit of the enzyme catalyzes the incorporation of 1 nmol of deoxyribonucleotides into polynucleotide in 10 min.

Production source

E.coli strain

Product Component	Amount
RTase, 10 U/µl	200 Units
10x RT-PCR buffer-SYBR Green dye: 500mM Tris-HCl (pH 8.3 @ 25°C), 150mM $(NH4)_2SO_4$, 15mM $MgCl_2$, 0.2% Tween20, 1mM DTT, 500 µg/ml BSA, with SYBR Green dye	1,000 µl
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Notes: *No* ROX passive reference dye is in the 10x RT-PCR buffers.

Ship and Store

The kit can be shipped at **4°C** (for up to 3 days).

It should be stored at **-20°C** for 24 months before use.

Set up 20µl of reaction

Component	Amount or final concentration
10x RT-PCR buffer	2 ul
dNTPs	Up to 200 uM
Target specific primers [a]	Each ≥0.1 uM
Taqman probe or SYBR Green [b]	Variable
RTase	1-2 U
Taq DNA polymerase [c]	1-2 U
RNA template [d]	As low as single digit copies of target RNA
Nuclease-free H_2O	To a total volume of 20 µl

[a] The primer T_m should be designed ≥60°C using primer3 software for high efficiency at the optimal temperature.

[b] TaqMan probe or SYBR Green dye can be used for fluorescent signal.

[c] Not included in the kit.

[d] RNA templates should be extracted by a qualified silica-based kit and eluted with low EDTA TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0-8.3).

Suggested thermo-cycling for RTase

Start reverse transcription at 60°C for 10 minutes, and then inactivate Thermophilic Reverse Transcriptase at 94-95°C for 2 minutes, followed by a PCR program.

Note: **Turn off ROX passive reference dye button** when setup assays on Applied Biosystems/ThermoFisher instruments.