WarmStart® RTx Reverse Transcriptase

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M0380S

50 reactions **15,000 U/ml** **Lot: 0011501**
RECOMBINANT **Store at –20°C** **Exp: 1/17**

Description: WarmStart RTx Reverse Transcriptase is a unique *in silico* designed RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well suited for use in LAMP (loop-mediated isothermal amplification). The WarmStart property enables high throughput applications, room temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

Source: An *E. coli* strain that carries the engineered RTx gene.

Application:

- RT-LAMP
- cDNA synthesis
- RT reactions requiring room temperature setup

Supplied in: 100 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, pH 7.4 @ 25°C

Reagents Supplied with Enzyme:
10X Isothermal Amplification Buffer, 100 mM $MgSO_4$

Reaction Conditions: 1X Isothermal Amplification Buffer, template, primer, dNTPs and 0.25–0.5 µl of WarmStart RTx Reverse Transcriptase in a reaction volume of 25 µl. Incubate at 50–55°C for cDNA synthesis or directly at 65°C for One-step RT-LAMP.

1X Isothermal Amplification Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
50 mM KCl
2 mM $MgSO_4$
0.1% Tween-20
pH 8.8 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 20 minutes at 50°C using poly(rA)•oligo(dT)18 as template.

Unit Assay Conditions: 20 mM Tris-HCl (pH 8.8), 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1% Tween-20, 0.5 mM dTTP, 0.4 mM poly(rA)•oligo(dT)18.

Heat Inactivation: 10 minutes at 80°C

Typical RT-LAMP Protocol

COMPONENT	FINAL CONC./AMT.
10X Isothermal Amplification Buffer	1X
$MgSO_4$ (100 mM)	6 mM (8 mM total)
dNTP Mix (10 mM)	1.4 mM
FIP/BIP Primers (25X)	1.6 µM
F3/B3 Primers (25X)	0.2 µM
LoopF/B Primers (25X)	0.4 µM
Bst 2.0 or *Bst* 2.0 WarmStart DNA Polymerase	8 units
WarmStart RTx Reverse Transcriptase	0.5 µl
Total Reaction Volume	25 µl

2. Mix by vortexing.
3. Place directly at 65°C.

Typical cDNA Synthesis Protocol:

COMPONENT	FINAL CONC./AMT.
10X Isothermal Amplification Buffer	1X
dNTP Mix (10 mM)	0.5 mM
Random Primer Mix	6 µM
RNase Inhibitor, Murine	20 units
WarmStart RTx Reverse Transcriptase	0.25 µl
Total Reaction Volume	20 µl

2. Mix by vortexing.
3. Incubate for 5 minutes at 25°C for annealing and 10 minutes at 55°C for synthesis.
4. Heat Inactivate WarmStart RTx Reverse Transcriptase by incubation at 80°C for 10 minutes.

Quality Control Assays

RNase Activity (Extended Digestion): A 10 µl reaction in 1X Isothermal Amplification Reaction Buffer containing 40 ng of labeled RNA and 15 units WarmStart RTx Reverse Transcriptase is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Endonuclease Activity (Nicking): A 50 µl reaction in 1X Isothermal Amplification Reaction Buffer containing 15 units WarmStart RTx Reverse Transcriptase with 1 µg ϕX174 RF I DNA for 4 hours at 37°C results in < 10% conversion to the RF II as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16-hour): A 50 µl reaction in 1X Isothermal Amplification Reaction Buffer containing 1 µg of Lambda DNA and 15 units of WarmStart RTx Reverse Transcriptase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release): A 50 µl reaction in 1X Isothermal Amplification Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and 15 units WarmStart RTx Reverse Transcriptase incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

Protein Purity (SDS-PAGE): WarmStart RTx Reverse Transcriptase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

Companion Products Sold Separately:

Random Primer Mix
#S1330S 100 µl

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

Deoxynucleotide Solution Set
#N0446S 25 µmol each

Deoxynucleotide Solution Mix
#N0447S 8 µmol each
#N0447L 40 µmol each

Bst 2.0 DNA Polymerase
#M0537S 1,600 units
#M0537L 8,000 units

Bst 2.0 WarmStart DNA Polymerase
#M0538S 1,600 units
#M0538L 8,000 units

Isothermal Amplification Buffer Pack
#B0537S 6.0 ml

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