

New England Biolabs Product Specification

Product Name:	WarmStart [®] RTx Reverse Transcriptase
Catalog #:	M0380S/L
Concentration:	15,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in 20 minutes at 50°C.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version:	PS-M0380S/L v2.0
Effective Date:	11 Jul 2018

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in Isothermal Amplification Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 15 units of RTx Reverse Transcriptase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

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

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 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.

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

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 15 units of WarmStart[®] RTx Reverse Transcriptase is screened for the presence of *E. coli* genomic DNA using SYBR[®] Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

RNase Activity Assay (4 Hour Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of WarmStart[®] RTx Reverse Transcriptase is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.



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Date 11 Jul 2018

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Director of Quality Control

