

## New England Biolabs Product Specification

<b>Product Name:</b>	WarmStart <sup>®</sup> RTx Reverse Transcriptase
<b>Catalog #:</b>	M0380S/L
<b>Concentration:</b>	15,000 units/ml
<b>Unit Definition:</b>	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.
<b>Shelf Life:</b>	24 months
<b>Storage Temp:</b>	-20°C
<b>Storage Conditions:</b>	10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)
<b>Specification Version:</b>	PS-M0380S/L v3.0
<b>Effective Date:</b>	12 Feb 2020

### 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 [<sup>3</sup>H] *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 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 15 units of WarmStart<sup>®</sup> 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<sup>®</sup> RTx Reverse Transcriptase is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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.



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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 <sup>®</sup> 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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