

## New England Biolabs Certificate of Analysis

*Product Name:* WarmStart<sup>®</sup> 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.  
*Lot #:* 0021612  
*Assay Date:* 12/2016  
*Expiration Date:* 12/2018  
*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 v1.0  
*Effective Date:* 10 Mar 2017

Assay Name/Specification (minimum release criteria)	Lot #0021612
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in Isothermal Amplification Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 15 units of WarmStart <sup>®</sup> RTx Reverse Transcriptase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in Isothermal Amplification Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 15 units of WarmStart <sup>®</sup> RTx Reverse Transcriptase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - 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 <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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - WarmStart <sup>®</sup> RTx Reverse Transcriptase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 15 units of WarmStart <sup>®</sup> RTx Reverse Transcriptase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR <sup>®</sup> Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.	<b>Pass</b>

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Assay Name/Specification (minimum release criteria)	Lot #0021612
RNase Activity Assay (4 Hour Digestion) - A 10 µl reaction in Isothermal Amplification Buffer 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.	<b>Pass</b>



Authorized by  
Karen Moreira  
10 Mar 2017



Inspected by  
Tony Spear-Alfonso  
06 Jan 2017

