

New England Biolabs Product Specification

<i>Product Name:</i>	<i>ProtoScript[®] II Reverse Transcriptase</i>
<i>Catalog #:</i>	<i>M0368S/L/X</i>
<i>Concentration:</i>	<i>200,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>20 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.01 % IGEPAL[®] CA-630, 50 % Glycerol, (pH 7.5 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0368S/L/X v1.0</i>
<i>Effective Date:</i>	<i>28 Jun 2016</i>

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in ProtoScript[®] II Reverse Transcriptase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 200 units of ProtoScript[®] II 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 ProtoScript[®] II Reverse Transcriptase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 200 units of ProtoScript[®] II 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 200 units of ProtoScript[®] II 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) - ProtoScript[®] II Reverse Transcriptase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 200 units of ProtoScript[®] II 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.



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Assay Name/Specification (minimum release criteria)

RNAse Activity Assay (4 Hour Digestion) - A 10 µl reaction in ProtoScript [®] II Reverse Transcriptase Reaction Buffer containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of ProtoScript [®] II 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.



Date 28 Jun 2016

Derek Robinson
Director of Quality Control

