New England Biolabs
Product Specification

Product Name: ProtoScript® II Reverse Transcriptase
Catalog #: M0368S/L/X
Concentration: 200,000 units/ml
Unit Definition: 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.
Shelf Life: 24 months
Storage Temp: -20°C
Storage Conditions: 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)
Specification Version: PS-M0368S/L/X v1.0
Effective Date: 28 Jun 2016

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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<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tr>
<td><strong>RNAse Activity Assay (4 Hour Digestion)</strong> - 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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</td>
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Derek Robinson  
Director of Quality Control  

Date  28 Jun 2016