New England Biolabs
Product Specification

Product Name: Q5® Reaction Buffer Pack
Catalog #: B9027S/V
Concentration: 5X Concentrate
Shelf Life: 36 months
Storage Temp: -20°C
Composition (1X): Proprietary
Specification Version: PS-B9027S v2.0
Effective Date: 12 Feb 2020

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking, Buffer) - A 50 µl reaction in 2X Q5® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 hour, Buffer) - A 50 µl reaction in 2X Q5® Reaction Buffer containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

PCR Amplification (20 kb Lambda DNA, Buffer) - A 50 µl reaction in Q5® Reaction Buffer in the presence of 200 µM dNTPs and 1 µM primers containing 10 ng Lambda DNA with 1 unit of Q5® High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.

PCR Amplification (7 kb Human Genomic DNA, Buffer) - A 50 µl reaction in Q5® Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.

Phosphatase Activity (pNPP, Buffer) - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl₂ containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 80 µl Q5® Reaction Buffer incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

qPCR DNA Contamination (E. coli Genomic, Buffer) - A minimum of 1 µl of Q5® Reaction Buffer 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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<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tr>
<td>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 Q5® Reaction Buffer is incubated at 37ºC. 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, Quality Control

Date 12 Feb 2020