

New England Biolabs Product Specification

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

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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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 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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Date 12 Feb 2020

Derek Robinson
Director, Quality Control

