**New England Biolabs**  
**Product Specification**

**Product Name:** Hemo KlenTaq® Reaction Buffer Pack  
**Catalog #:** B0332S  
**Concentration:** 5X Concentrate  
**Shelf Life:** 36 months  
**Storage Temp:** -20°C  
**Composition (1X):**  
60 mM Tricine, 5 mM (NH₄)₂SO₄, 3.5 mM MgCl₂, 6% Glycerol, (pH 8.7 @ 25°C)  
**Specification Version:** PS-B0332S v1.0  
**Effective Date:** 08 Aug 2016

**Assay Name/Specification (minimum release criteria)**

**Endonuclease Activity (Nicking, Buffer)** - A 50 µl reaction in 2X Hemo KlenTaq® 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 Hemo KlenTaq® Reaction Buffer containing 1 µg of T3 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 (0.5 kb Whole Blood DNA, Buffer)** - A 50 µl reaction in the presence of 200 µM dNTPs and 0.3 µM primers containing 10% whole blood treated with sodium heparin, sodium EDTA, potassium EDTA or sodium citrate with 4 µl Hemo KlenTaq® and 1X Hemo KlenTaq® Reaction Buffer for 35 cycles of PCR amplification results in the expected 0.5 kb product.

**pH (buffers/solutions)** - The pH of 5X Hemo KlenTaq® Reaction Buffer is between pH 8.6 and 8.8 at 25°C.

**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 Hemo KlenTaq® 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 Hemo KlenTaq® 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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<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tbody>
<tr>
<td>RNase Activity (Extended 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 Hemo KlenTaq® Reaction Buffer 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 08 Aug 2016