

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>Diluent B (with rAlbumin)</i>
<i>Catalog #:</i>	<i>B8533S</i>
<i>Concentration:</i>	<i>1X Concentrate</i>
<i>Shelf Life:</i>	<i>36 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Composition (1X):</i>	<i>10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 500 µg/ml rAlbumin, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-B8533S v1.0</i>
<i>Effective Date:</i>	<i>11 Aug 2023</i>

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

**Endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 µl of Diluent B (with rAlbumin) 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)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of PhiX174-HaeIII DNA and a minimum of 10 µl of Diluent B (with rAlbumin) incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**pH (buffers/solutions)** - The pH of 1X Diluent B (with rAlbumin) is between pH 7.3 and 7.5 at 25°C.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 1 µl of Diluent B (with rAlbumin) 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.

**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 Diluent B (with rAlbumin) is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

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