240 County Road Ipswich, MA 01938-2723

Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Product Specification

Product Name: Diluent A
Catalog #: B8001S

Concentration: 1X Concentrate
Shelf Life: 36 months
Storage Temp: -20°C

Composition (1X): 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml BSA, 50 % Glycerol, (pH 7.4 @ 25°C)

Specification Version: PS-B8001S v1.0
Effective Date: 15 May 2018

## Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in CutSmart® Buffer containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 10  $\mu$ l of Diluent A 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  $\mu$ l reaction in CutSmart® Buffer containing 1  $\mu$ g of PhiX174-HaeIII DNA and a minimum of 10  $\mu$ l of Diluent A 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 A is between pH 7.3 and 7.5 at 25°C.

qPCR DNA Contamination (E. coli Genomic) - A minimum of 1  $\mu$ l of Diluent A 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  $\leq 1$  E. coli genome.

RNase Activity (Extended Digestion) - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1  $\mu$ l of Diluent A 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.

Derek Robinson

Director of Quality Control





