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## New England Biolabs Certificate of Analysis

| Product Name:          | Diluent A  |
|------------------------|--|
| Catalog #:             | B8001S   |
| Concentration:         | 1X Concentrate   |
| <i>Lot</i> #:          | 0011703  |
| Assay Date:            | 03/2017  |
| Expiration Date:       | 03/2020  |
| 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)  | Lot #0011703 |
|--|--------------|
| <b>Endonuclease Activity (Nicking)</b> - 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.  | Pass         |
| <b>Non-Specific DNase Activity (16 Hour)</b> - A 50 $\mu$ l reaction in CutSmart® Buffer containing 1 $\mu$ g of PhiX174-<br>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<br>detectable nuclease degradation as determined by agarose gel electrophoresis.  | Pass         |
| <b>pH (buffers/solutions)</b> - The pH of 1X Diluent A is between pH 7.3 and 7.5 at 25°C.  | Pass         |
| <b>qPCR DNA Contamination (E. coli Genomic)</b> - A minimum of 1 $\mu$ l of Diluent A is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is $\leq 1$ <i>E. coli</i> genome. | Pass         |
| <b>RNase Activity (Extended Digestion)</b> - A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-<br>stranded RNA and a minimum of 1 $\mu$ l of Diluent A is incubated at 37°C. After incubation for 16 hours, >90% of<br>the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.   | Pass         |

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Authorized by Derek Robinson 15 May 2018



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Inspected by Tony Spear-Alfonso 30 Mar 2017