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## New England Biolabs Certificate of Analysis

Product Name: Nuclease-free Water

Catalog Number: B1500L
Lot Number: 10019500
Expiration Date: 11/2019
Storage Temperature: 25°C

Specification Version: PS-B1500S/L v2.0

| Nuclease-free Water Component List |                       |            |                      |  |
|------------------------------------|-----------------------|------------|----------------------|--|
| <b>NEB Part Number</b>             | Component Description | Lot Number | Individual QC Result |  |
| B1500SVIAL                         | Nuclease-free Water   | 0021711    | Pass                 |  |

| Assay Name/Specification   | Lot # 10019500 |
|--|----------------|
| Endonuclease Activity (Nicking, Water) A 50 μl reaction in CutSmart® Buffer containing 1 μg of supercoiled PhiX174 RF I DNA with Nuclease-free Water incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.                                   | Pass           |
| Endotoxin Testing (Endosafe®) Each test channel of the cartridge is loaded with 25 µl of Nuclease-free Water, then placed into the Endosafe MCS reader for analysis resulting in a measurement of <0.01 EU/ml.   | Pass           |
| Non-Specific DNase Activity (16 Hour, Water) A 50 µl reaction in CutSmart® Buffer containing 1 µg of PhiX174-HaeIII DNA with Nuclease-free Water incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.                   | Pass           |
| RNase Activity (Extended Digestion, Water) A 10 µl reaction in 1X NEBuffer 4 containing 40 ng of RNA transcript with Nuclease-free Water is incubated at 37°C. After incubation for 16 hours, no detectable degradation of the RNA is observed as determined by gel electrophoresis using fluorescent detection. | Pass           |
| UV-Visable Scan A UV-Visible scan using a spectrophotometer that covers the range of 200nm to 800nm will have no detectable peaks above background.  | Pass           |
| qPCR DNA Contamination (E. coli Genomic, Water)  | Pass           |



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|--|----------------|
| Nuclease-free Water is used to make a qPCR master mix and screened across a 96 well plate for the presence of E. coli genomic DNA using 40 cycles of SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Melt curve analysis results in |                |
| < 5% positive samples above background.  |                |

This product has been tested and shown to be in compliance with all specifications.

Michael Dalton

Production Scientist 15 Aug 2018 Nick Privitera

Packaging Quality Control Inspector

15 Aug 2018