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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Certificate of Analysis

| Product Name: | Isothermal Amplification Buffer |
|------------------------|---|
| Catalog Number: | B0537S |
| Concentration: | 10 X Concentrate |
| Lot Number: | 10036060 |
| Expiration Date: | 11/2020 |
| Storage Temperature: | -20°C |
| Specification Version: | PS-B0537S v1.0 |
| Composition (1X): | 20 mM Tris-HCl, 50 mM KCl, 10 mM (NH4)2SO4, 2 mM MgSO4, 0.1 % Tween® 20, (pH 8.8 @ 25°C) |

| Isothermal Amplification Buffer Component List | | | |
|--|---------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| B0537SVIAL | Isothermal Amplification Buffer | 0031711 | Pass |

| Assay Name/Specification | Lot # 10036060 |
|--|----------------|
| Endonuclease Activity (Nicking, Buffer) A 50 μ I reaction in 2X Isothermal Amplification Buffer containing 1 μ g 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. | Pass |
| Non-Specific DNase Activity (16 hour, Buffer) A 50 µl reaction in 2X Isothermal Amplification 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. | Pass |
| pH (buffers/solutions) The pH of 10X Isothermal Amplification Buffer is between pH 8.7 and 8.9 at 25°C. | Pass |
| 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 40 µl Isothermal Amplification Buffer incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis. | Pass |
| qPCR DNA Contamination (E. coli Genomic, Buffer) A minimum of 1 μl of Isothermal Amplification Buffer is screened for the presence of | Pass |





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| 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 Assay (4 Hour Digestion) A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 μ l of Isothermal Amplification Buffer is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection. | Pass |

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

hästie Vazanez

Christie Vazquez Production Scientist 31 Jan 2019

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Michael Tonello Packaging Quality Control Inspector 31 Jan 2019

