NEW ENGLAND BioLabs Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*Isothermal Amplification Buffer Pack*
Catalog #:	*B0537S*
Concentration:	*10X Concentrate*
Lot #:	*0031711*
Assay Date:	*11/2017*
Expiration Date:	*11/2020*
Storage Temp:	*-20°C*
Composition (1X):	*20 mM Tris-HCl, 50 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1 % Tween® 20, (pH 8.8 @ 25°C)*
Specification Version:	*PS-B0537S v1.0*
Effective Date:	*14 Nov 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0031711**
Endonuclease Activity (Nicking, Buffer) - A 50 µl reaction in 2X Isothermal Amplification Buffer containing 1 µg 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 $MgCl_2$ 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 *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.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0031711**
RNAse Activity Assay (4 Hour 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 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**

Authorized by
Lynne Apone
14 Nov 2017

Inspected by
Tony Spear-Alfonso
06 Dec 2017

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices