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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
Packaging Lot Number:	10055980
Expiration Date:	02/2022
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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
B0537SVIAL	Isothermal Amplification Buffer	10035085	Pass	

Assay Name/Specification	Lot # 10055980
<b>Endonuclease Activity (Nicking, Buffer)</b> A 50 $\mu$ I reaction in 2X Isothermal Amplification Buffer containing 1 $\mu$ 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
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> 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
<b>pH (buffers/solutions)</b> The pH of 10X Isothermal Amplification Buffer is between pH 8.7 and 8.9 at 25°C.	Pass
<b>Phosphatase Activity (pNPP, Buffer)</b> 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
<b>qPCR DNA Contamination (E. coli Genomic, Buffer)</b> A minimum of 1 μl of Isothermal Amplification Buffer is screened for the presence of	Pass





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Assay Name/Specification	Lot # 10055980
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.	
<b>RNAse Activity Assay (4 Hour Digestion)</b> 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 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.

Diffeen Duquette

Production Scientist 21 Feb 2019

Josh Hersey Packaging Quality Control Inspector 22 Oct 2019

