

## New England Biolabs Certificate of Analysis

**Product Name:** Isothermal Amplification Buffer II Pack  
**Catalog #:** B0374S  
**Concentration:** 10X Concentrate  
**Lot #:** 0031711  
**Assay Date:** 11/2017  
**Expiration Date:** 11/2020  
**Storage Temp:** -20°C  
**Composition (1X):** 20 mM Tris-HCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 150 mM KCl, 2 mM MgSO<sub>4</sub>, 0.1 % Tween® 20, (pH 8.8 @ 25°C)  
**Specification Version:** PS-B0374S v2.0  
**Effective Date:** 14 Nov 2017

Assay Name/Specification (minimum release criteria)	Lot #0031711
<b>Endonuclease Activity (Nicking, Buffer)</b> - A 50 µl reaction in 2X Isothermal Amplification Buffer II 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.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> - A 50 µl reaction in 2X Isothermal Amplification Buffer II 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.	<b>Pass</b>
<b>pH (buffers/solutions)</b> - The pH of 10X Isothermal Amplification Buffer II is between pH 8.7 and 8.9 at 25°C.	<b>Pass</b>
<b>Phosphatase Activity (pNPP, Buffer)</b> - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl <sub>2</sub> containing 2.5 mM <i>p</i> -Nitrophenyl Phosphate (pNPP) and a minimum of 40 µl 10X Isothermal Amplification Buffer II incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	<b>Pass</b>
<b>qPCR DNA Contamination (E. coli Genomic, Buffer)</b> - A minimum of 1 µl of Isothermal Amplification Buffer II 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 ≤ 1 <i>E. coli</i> genome.	<b>Pass</b>
<b>RNase Activity Assay (4 Hour Digestion)</b> - 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 II incubated for 4 hours at 37°C results in no detectable degradation of the RNA as determined by gel electrophoresis using fluorescent detection.	<b>Pass</b>



Authorized by  
Lynne Apone  
14 Nov 2017



Inspected by  
Tony Spear-Alfonso  
04 Dec 2017

