

## New England Biolabs Product Specification

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|-------------------------------|---|
| <i>Product Name:</i>          | <i>Isothermal Amplification Buffer II Pack</i>  |
| <i>Catalog #:</i>             | <i>B0374S</i>   |
| <i>Concentration:</i>         | <i>10X Concentrate</i>  |
| <i>Shelf Life:</i>            | <i>36 months</i>  |
| <i>Storage Temp:</i>          | <i>-20°C</i>  |
| <i>Composition (1X):</i>      | <i>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<sup>®</sup> 20, (pH 8.8 @ 25°C)</i> |
| <i>Specification Version:</i> | <i>PS-B0374S v2.0</i>   |
| <i>Effective Date:</i>        | <i>15 Feb 2017</i>  |

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking, Buffer)** - 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.

**Non-Specific DNase Activity (16 hour, Buffer)** - 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.

**pH (buffers/solutions)** - The pH of 10X Isothermal Amplification Buffer II is between pH 8.7 and 8.9 at 25°C.

**Phosphatase Activity (pNPP, Buffer)** - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl<sub>2</sub> containing 2.5 mM *p*-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.

**qPCR DNA Contamination (*E. coli* Genomic, Buffer)** - A minimum of 1 µl of Isothermal Amplification Buffer II is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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.

**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 II incubated for 4 hours at 37°C results in no detectable degradation of the RNA as determined by gel electrophoresis using fluorescent detection.



Date 15 Feb 2017

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Director of Quality Control

