NEW ENGLAND BioLabs Inc.

240 County Road
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New England Biolabs
Certificate of Analysis

Product Name:	*Antarctic Phosphatase*
Catalog #:	*M0289S/L*
Concentration:	*5,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will dephosphorylate 1 µg of pUC19 vector DNA cut with a restriction enzyme generating 5´ recessed ends in 30 minutes at 37°C. Dephosphorylation is defined as >95% inhibition of recircularization in a self-ligation reaction and is measured by transformation into E. coli.*
Lot #:	*0371702*
Assay Date:	*02/2017*
Expiration Date:	*2/2019*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 50 % Glycerol, (pH 7.4 @ 25°C)*
Specification Version:	*PS-M0289S/L v1.0*
Effective Date:	*22 Feb 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0371702**
Endonuclease Activity (Nicking) - A 50 µl reaction in Antarctic Phosphatase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of Antarctic Phosphatase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in Antarctic Phosphatase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 50 units of Antarctic Phosphatase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 4 containing 1 µg of PhiX174-HaeIII DNA and a minimum of 50 units of Antarctic Phosphatase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Protein Purity Assay (SDS-PAGE) - Antarctic Phosphatase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 5 units of Antarctic Phosphatase 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 #0371702**
RNase Activity (Extended 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 Antarctic Phosphatase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**

Authorized by
Derek Robinson
22 Feb 2017

Inspected by
Ana Egana
28 Feb 2017