

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Product Specification

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 μ g 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.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	10 mM Tris-HCl, 1 mM MgCl2, 0.01 mM ZnCl2, 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version:	<i>PS-M0289S/L v2.0</i>
Effective Date:	23 Oct 2019

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 μ l reaction in CutSmart® Buffer containing 1 μ g 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.

Exonuclease Activity (Radioactivity Release) - A 50 μ l reaction in Antarctic Phosphatase Reaction Buffer containing 1 μ g of a mixture of single and double-stranded [³H] *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.

Non-Specific DNase Activity (16 Hour) - A 50 μ l reaction in NEBuffer 4 containing 1 μ g 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.

Protein Purity Assay (SDS-PAGE) - Antarctic Phosphatase is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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.



PS-M0289S/L v2.0 Page 1 of 2



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Assay Name/Specification (minimum release criteria)

RNase Activity (Extended Digestion) - A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 μ l 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.

Date 23 Oct 2019

Derek Robinson Director of Quality Control



PS-M0289S/L v2.0 Page 2 of 2