

## 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<sub>2</sub>, 0.01 mM ZnCl<sub>2</sub>, 50 % Glycerol, (pH 7.4 @ 25°C)  
*Specification Version:* PS-M0289S/L v1.0  
*Effective Date:* 22 Feb 2017

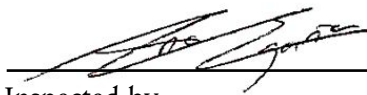
Assay Name/Specification (minimum release criteria)	Lot #0371702
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in Antarctic Phosphatase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of Antarctic Phosphatase incubated for 4 hours at 37°C results in <10%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

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<b>Assay Name/Specification</b> (minimum release criteria)	<b>Lot #0371702</b>
<b>RNase Activity (Extended 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 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.	<b>Pass</b>



Authorized by  
Derek Robinson  
22 Feb 2017



Inspected by  
Ana Egana  
28 Feb 2017

