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New England Biolabs Certificate of Analysis

Product Name: Antarctic Phosphatase

Catalog Number: M0289L Concentration: 5,000 U/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.

Packaging Lot Number: 10087266
Expiration Date: 12/2022
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 1 mM MgCl2, 0.01 mM ZnCl2, 50 % Glycerol, (pH 7.4 @

25°C)

Specification Version: PS-M0289S/L v2.0

| Antarctic Phosphatase Component List | | | | | |
|--------------------------------------|---------------------------------------|------------|----------------------|--|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | | |
| M0289LVIAL | Antarctic Phosphatase | 10087265 | Pass | | |
| B0289SVIAL | Antarctic Phosphatase Reaction Buffer | 10081109 | Pass | | |

| Assay Name/Specification | Lot # 10087266 |
|--|----------------|
| 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 |
| 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 | Pass |



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| Assay Name/Specification | Lot # 10087266 |
|--|----------------|
| Coomassie Blue detection. | |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in Antarctic Phosphatase Reaction Buffer containing 1 µg 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. | Pass |
| 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 |
| Endonuclease Activity (Nicking) A 50 µl reaction in CutSmart® 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 |

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Ana Egana **Production Scientist** 29 Jan 2021

Michael Tonello

Packaging Quality Control Inspector

29 Jan 2021



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