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New England Biolabs Product Specification

Product Name:	RNA 5' Pyrophosphohydrolase (RppH)
Catalog #:	M0356S
Concentration:	5,000 units/ml
Unit Definition:	One unit is the amount of enzyme that converts 1 μg 300 mer RNA transcript into a XRN-1 digestible RNA in 30 minutes at 37°C.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	200 mM NaCl, 20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.01% Triton®X-100, (pH 7.5 @ 25° C)
Specification Version:	PS-M0356S v1.0
Effective Date:	16 Nov 2017

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 μ l reaction in NEBuffer 2 containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 25 units of RNA 5' Pyrophosphohydrolase (RppH) 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 NEBuffer 2 containing 1 μ g of a mixture of single and doublestranded [³H] *E. coli* DNA and a minimum of 25 units of RNA 5' Pyrophosphohydrolase (RppH) incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 25 units of RNA 5' Pyrophosphohydrolase (RppH) incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity Assay (SDS-PAGE) - RNA 5' Pyrophosphohydrolase (RppH) is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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 5 units of RNA 5' Pyrophosphohydrolase (RppH) is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 16 Nov 2017

Derek Robinson Director of Quality Control



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