

## New England Biolabs Certificate of Analysis

*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.  
*Lot #:* 0021707  
*Assay Date:* 07/2017  
*Expiration Date:* 07/2019  
*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)	Lot #0021707
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in NEBuffer 2 containing 1 µg 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.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in NEBuffer 2 containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> 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.	<b>Pass</b>
<b>Phosphatase Activity (pNPP)</b> - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl <sub>2</sub> containing 2.5 mM <i>p</i> -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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - RNA 5' Pyrophosphohydrolase (RppH) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>RNase Activity Assay (4 Hour Digestion)</b> - 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.	<b>Pass</b>



Authorized by  
Derek Robinson  
16 Nov 2017



Inspected by  
Bhairavi Jani  
25 Jul 2017

