RNA 5´ Pyrophosphohydrolase (RppH)

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M0356S 001130513051

M0356S

RR NEB2

200 units	**5,000 U/ml**	**Lot: 0011305**
RECOMBINANT	**Store at –20°C**	**Exp: 5/15**

Description: The bacterial RNA 5´ Pyrophosphohydrolase (RppH) removes pyrophosphate from the 5´ end of triphosphorylated RNA to leave a 5´ monophosphate RNA (1). The RppH protein was also known as NudH/YgdP which can split Ap_5A to ADP and ATP (2).

Source: An *E. coli* strain containing a clone of the *E. coli* RppH gene.

Supplied in: 200 mM NaCl, 0.1 mM EDTA, 20 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.01% Triton X-100 and 50% glycerol.

Applications:

- Convert RNA transcript (ppp-RNA) to 5´ monophosphate RNA (p-RNA) for 5´ RNA ligation or for removal by XRN-1.
- RNA 5´ end structure analysis.

Reagents Supplied with Enzyme:
10X NEBuffer 2

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 25°C

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.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 5 units of RppH with 40 ng of 300 mer RNA transcript for 4 hours at 37°C resulted in less than 10% degradation of RNA as determined by denaturing PAGE analysis.

Endonuclease Assay: Incubation of a 10 µl reaction containing 5 units of RppH with 300 ng of supercoiled plasmid for 4 hours at 37°C produced less than 10% nicked or linear molecules as determined by agarose gel electrophoresis.

Exonuclease Assay: Incubation of a 50 µl reaction containing 25 units of RppH with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.5% of the total radioactivity.

Phosphatase Activity: Incubation of a 100 µl reaction containing 25 units of RppH with 4-nitrophenyl phosphate under standard conditions (NEBuffer 2) at 37°C for 4 hours, no phosphatase activity was detected.

Notes On Use:
Since RppH is active in the presence of Mg^{2+}, it can be incubated with RNA ligase or XRN-1.

References:

1. Deana, A. et al. (2008) *Nature,* 451, 355–358.
2. Bessman, M.J. et al (2001) *JBC,* 276, 37834.

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CERTIFICATE OF ANALYSIS