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

**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 ADP and ATP (2).

**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

**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.

**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.
- 5′ Pyrophosphohydrolase

**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 [3H] *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 Mg2+, it can be incubated with RNA ligase or XRN-1.

**References:**