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RNA 5' Pyrophosphohydrolase (RppH)

Cat. No.	size
E1280-01	200 units

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/b2830/JW2798 which can split Ap₅A to ADP and ATP (2). Could be used as alternative for TAP.

Source: An E. coli strain containing a cloned RppH gene from E. coli.

Format: liquid

Concentration: 5 000 units/ml

Unit Definition: One unit is the amount of enzyme that converts 1 μ g 300 mer RNA transcript into a XRN-1 digestible RNA in 30 minutes at 37°C.

Storage Conditions: Store at -20°C.

Quality Control:

All preparations are assayed for contaminating endonucleases, exonucleases, nonspecific RNases, single- and doublestranded DNase activities. Greater than 90% as determined by SDS-PAGE.

References:

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

Storage Buffer: 500 mM NaCl, 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) Tergitol™ TMN, 50% glycerol.

RppH Dephosphorylation Buffer (1x): 10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT, pH 7.9 at 25°C.

RppH Decapping Buffer (1x): 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM MgSO₄, 0.1% (v/v) TergitolTM TMN, pH 8.8 at 25°C.

Removal of pyrophosphate from 5' triphosphorylated RNA (20 µl):

Component:	Amount:
10 x RppH Dephosphorylation Buffer	2 μl
5' triphosphorylated RNA	Up to 2 µg
RNA 5´ Pyrophosphohydrolase (RppH)	10-20 U
RNase-free Water	to 20 μl

Decapping eukaryotic mRNA with RppH (50 μl):

Component:	Amount:
10 x RppH Decapping Buffer	5 μl
RNA	5-500 ng
RNA 5´ Pyrophosphohydrolase (RppH)	5-25 U
RNase-free Water	to 50 μl

Incubate at 37°C for 30-60 minutes, proceed to RNA clean up for further RNA manipulation steps.