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RNA 5′ Polyphosphatase

Cat. No. RP8092H

** Patent pending*

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1. Introduction

RNA 5′ Polyphosphatase* is a Mg^{2+}-independent, 19,000-kDa phosphohydrolase discovered and characterized by Epicentre scientists. The enzyme sequentially removes the γ and β phosphates from 5′-triphosphorylated RNA (primary RNA transcripts) and 5′-diphosphorylated RNA.

5′ pppN--------OH 3′ or 5′ ppN--------OH 3′

↓

5′ pN----------OH 3′ + 2 P_i

RNA 5′ Polyphosphatase has no activity on RNA with a 5′ cap (e.g., 5′ m^7GpppN-----OH 3′) or a 5′-monophosphorylated (5′ pN-----OH 3′) end. However, NTPs and dNTPs are used as substrates yielding the corresponding NMPs and dNMPs + inorganic phosphate.

(d)NTP → (d)NMP + 2 P_i

RNA 5′ Polyphosphatase is available in a 200 Unit size at a concentration of 20 U/µl. A 10X Reaction buffer is provided with the enzyme.

Applications

- Conversion of 5′-triphosphorylated RNA to 5′-monophosphorylated RNA for use in 5′-T4 RNA Ligase-mediated RNA "tagging" strategies.
- Analysis of 5′-end structure of RNA.
- Preparation of substrate RNA molecules for subsequent degradation using Epicentre's Terminator™ 5′-Phosphate-Dependent Exonuclease.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: RNA 5′ Polyphosphatase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol, 0.1 mM EDTA and 0.1% Triton® X-100.

Unit Definition: 1 Unit of RNA 5′ Polyphosphatase results in the release of 1 nmol of inorganic phosphate from ATP in 1 hour at 37°C under standard assay conditions.

RNA 5′ Polyphosphatase 10X Reaction Buffer: 0.5 M HEPES-KOH (pH 7.5), 1 M NaCl, 10 mM EDTA, 1% β-mercaptoethanol, and 0.1% Triton X-100.

Contaminating Activity Assays: RNA 5′ Poly-phosphatase is free of detectable exo- and endonuclease and RNase activities.

Enzyme Inhibitors: RNA 5′ Polyphosphatase activity is inhibited approximately 50% by 100 µM inorganic phosphate, using ATP as a substrate.

3. Related Products

The following products are also available:

- RiboGuard™ RNase Inhibitor
- Tobacco Acid Pyrophosphatase
- Terminator™ 5′-Phosphate-Dependent Exonuclease
- T4 RNA Ligase

4. RNA Preparation

1. The RNA should be dissolved in RNase-free Water or RNase-free TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA). Total RNA or fractionated RNA preparations can be used.
2. RNA 5′ Polyphosphatase activity is inhibited approximately 50% by 100 µM inorganic phosphate, using ATP as a substrate. Care should be taken to purify away any residual reaction components so as not to carry over inorganic phosphate into the RNA 5′ Polyphosphatase reaction.
3. rNTPs, rNDPs, dNTPs, and dNDPs are all substrates for RNA 5′ Polyphosphatase. Therefore, it is strongly recommended that any (deoxy)nucleotide tri- or di-phosphates be removed from the RNA sample before use.

RNA 5′ Polyphosphatase Standard Protocol

1. Thaw and thoroughly mix the RNA 5′ Polyphosphatase 10X Reaction Buffer prior to use.
2. Combine the following components:

x	µl	RNase-Free water
2	µl	RNA 5′ Polyphosphatase 10X Reaction Buffer
0.5	µl	RiboGuard RNase Inhibitor (optional)
x	µl	RNA sample (up to 5 µg) (see RNA Preparation)
1	µl	RNA 5′ Polyphosphatase (20 Units)
20	µl	Total reaction volume

3. Gently but thoroughly mix the reaction.
4. Incubate at 37°C for 30 minutes.
5. Purify the treated RNA by a method appropriate to the downstream application.

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