Manual

RNA 5′ Polyphosphatase

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RNA 5′ Polyphosphatase is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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GENOMIC ANALYSIS BY LGC

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1. Introduction

RNA 5′ Polyphosphatase* is a Mg^{2+}-independent, 19,000-kDa phosphohydrolase. 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′ m7GpppN-----OH 3′), a 5′-monophosphorylated end (5′ pN-----OH 3′) or a 5′-hydroxyl end (5′ HO OH 3′). 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 400 unit size at a concentration of 20 U/µL. A 10X Reaction buffer is provided with the enzyme.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
RNA 5′ Polyphosphatase	400 units	RP8092H	RNA 5′ Polyphosphatase (20 unit/µL)	E0113-20D1	20 µL
			RNA 5′ Polyphosphatase 10X Reaction Buffer	SS000807-D1	100 µL

3. 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 (Rohm & Haas).

Unit definition: One 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′ Polyphosphatase 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.

4. 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 Terminator™ 5′-Phosphate-Dependent Exonuclease (Biosearch Technologies).

5. 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)
y	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.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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