Enzymax LLC
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Product Information

Product Name: RNA polyphosphatase
Catalog #: 97
Size: 200 units (20 U/µl)

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Product: RNA polyphosphatase

Product Description:

RNA polyphosphatase removes gamma and beta phosphates from triphosphorylated RNA. It can be used to clone triphosphorylated RNAs, such as certain endogenous small RNAs and nascent transcripts. Unlike other commercially available RNA polyphosphase (such as RNA 5' Polyphosphatase), this enzyme works well in the broad range of temperature and reaction buffers including RNA ligation buffer, therefore RNA ligase and RNA polyphosphatase can be mixed together during 5'RACE or cloning of triphosphorylated RNAs. This indeed simplifies the cloning protocol.

Components:

10 µl RNA polyphosphatase: 20 U/µl 10 X RP reaction buffer : 0.5 M Tris, pH7.5, 0.1 M $MgCl_2$, 0.1% BSA, 0.1 M DTT

Unit definition:

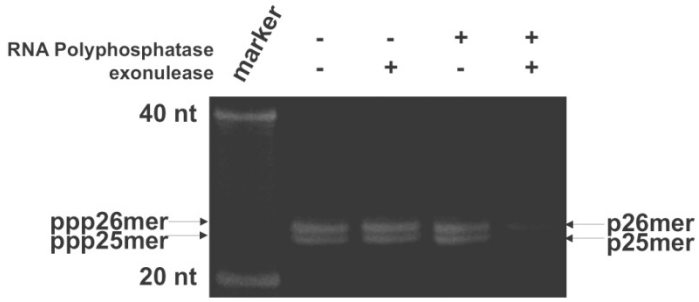
1U of RNA polyphosphatase can convert at least 2 pmole pppRNA to pRNA.

Procedure:

Assemble the following components in a clean DNAase/RNase free micro-centrifuge tube:

pppRNA (10 µM)	1 µl
RNA polyphosphatase (20U/µl)	0.25 µl
10 X RP reaction buffer	1 µl
H2O	7.25 µl

Incubate the tube at room temperature or 30°C for 1 hour.
Note: This protein also works well at 16 °C.



RNA polyphosphatase converts pppRNA to pRNA

Note for the gel above:

1. pRNA (p26mer and p25mer) were degraded by exonuclease, while pppRNA (both 25 and 26mer) were resistant to exonuclease digestion.
2. pRNA (lane 3 and 4 from marker) runs slightly faster than pppRNA (Lane 1 and 2 from marker)

Note:

1. This enzyme can be inactivated by incubation at 68 °C for 15 minutes.
2. RNA samples should be EDTA-free.
3. RNase inhibitor may be needed if samples have RNases contamination. However, if possible, please don't use RNase inhibitors when treating with a tiny amount of RNAs (for example 1 pmole), since RNase inhibitor may actually contain certain RNases.
4. This enzyme works in common RNA ligation buffer at low temperature. Therefore it can be mixed with RNA ligase during 5' RACE or Cloning of triphosphorylated RNAs. This significantly simplifies the protocols. We do have 5'RACE Kit (**EZ-RLM5'RACE Kit**) and Small RNA library kit available (**EZNext RNAseq library Kit**), which are easy (single tube preparation), low cost, and work for different types of RNAs.
5. More enzymes may be needed for long RNA substrates.