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Tobacco Acid Pyrophosphatase

Cat. Nos. T19050, T19100, T19250, T19500, and T81050

1. Introduction

Tobacco Acid Pyrophosphatase (TAP) hydrolyzes various pyrophosphate bonds, including those in adenosine triphosphate (ATP), cyclic nucleotides, and dinucleotides, but not those in RNA or DNA.[1] In addition, the enzyme cleaves the pyrophosphate bond of the 5′-terminal methylated guanine nucleotide "cap" of eukaryotic messenger RNAs.[2] The cap structure is also found on many small nuclear RNAs (snRNAs), heterogeneous nuclear RNAs, and many viral RNAs. Complete hydrolysis results in removal of the β and γ phosphates, leaving only the α phosphate attached. The resulting 5′-monophosphorylated terminus may be ligated to a 3′-hydroxylated terminus using T4 RNA Ligase or dephosphorylated with APex™ Heat-Labile Alkaline Phosphatase for end labeling.

TAP is available in 50-, 100-, 250-, and 500-Unit sizes at a concentration of 10 U/µl. The 50-Unit size is also available at 5 U/µl. A 10X TAP Reaction Buffer is provided with the enzyme.

2. Applications

Cloning of Full-Length cDNAs. Rapid amplification of cDNA ends (RACE) is a method whereby unknown sequences 5′ and 3′ of a known sequence are obtained through reverse transcription, followed by amplification and cloning.[3] RNA is decapped and an oligonucleotide anchor sequence is ligated to the 5′ end using T4 RNA Ligase.

Radiolabeling RNA.[4] Following removal of the cap structure with TAP and dephosphorylation with APex Phosphatase, RNA can be end-labeled with T4 Polynucleotide Kinase and γ-[^{32}P]-ATP and used for sequencing or as a hybridization probe.

RNA 5′-End Conversion. In addition to decapping capped mRNA, TAP can also be used to convert 5′-triphosphate RNA into 5′-monophosphate RNA.

3. Product Specifications

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

Storage Buffer: TAP is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1.0 mM dithiothreitol, 0.1 mM EDTA, and 0.01% Triton® X-100.

Unit Definition: One unit releases 1 nmol of inorganic phosphate from m^{7}GpppG in 30 minutes at 37°C under standard assay conditions.

Note: *One Epicentre* m^{7}GpppG *Unit is approximately equivalent to 15 Epicentre ATPase Units.*

10X TAP Reaction Buffer: 500 mM sodium acetate (pH 6.0), 10 mM EDTA, 1% β-mercaptoethanol, and 0.1% Triton X-100.

Contaminating Activity Assays: TAP is free of detectable RNase activity as judged by incubation of 1 µg of an SP6 transcript with 50 units of TAP for 1 hour at 37°C, followed by agarose gel electrophoresis. The enzyme is also free of detectable exonuclease and endonuclease activities.

4. Suggested Protocols

For Ligation of Oligonucleotides to RNA: Perform Part A; proceed to the ligation reaction.

For End Labeling of RNA: Perform Parts A, B, and C.

4.A. Removal of the Cap from RNA

1. Assemble the reaction in a microcentrifuge tube on ice in the order given (see Note).

x µl	Nuclease-Free Water
25 pmol	Capped RNA
5 µl	10X TAP Reaction Buffer
25 U	TAP
50 µl	Total reaction volume

2. Incubate at 37°C for 1-2 hours.

   Note: *TAP does not require ATP for activity. m^7GpppG is likewise not required for enzyme activity, but is used as a substrate in the measurement of enzyme activity. One picomole of a 1-kb RNA is approximately equivalent to 330 ng.*

4.B. Removal of the α Phosphate from RNA

1. Equilibrate the reaction from Part 4.A at 30°C and assemble the following reaction:

50 µl	TAP-treated RNA reaction mixture from Part 4.A
38 µl	Nuclease-Free Water
10 µl	10X APex Alkaline Phosphatase Buffer
2 µl	APex Alkaline Phosphatase
100 µl	Total reaction volume

2. Incubate at 37°C for 1 hour.
3. Inactivate the APex Phosphatase by extracting with an equal volume of phenol:chloroform (1:1, v/v). Transfer the aqueous phase to a clean tube. Re-extraction of the organic phase with buffer may improve recovery of the RNA. Extract the combined aqueous phases with an equal volume of chloroform. Ethanol-precipitate and resuspend the RNA in sterile Nuclease-Free Water.

4.C. End-Labeling Dephosphorylated RNA with T4 Polynucleotide Kinase (T4 PNK)

1. Assemble the reaction in a microcentrifuge tube on ice in the order given.

25 pmol	dephosphorylated RNA
25 pmol	γ-[^{32}P]-ATP (3,000-7,000 Ci/mmol)
x µl	10X T4 PNK Buffer
5-10 U	T4 PNK

2. Incubate at 37°C for 30 minutes.
3. Inactivate the T4 PNK by heating the reaction mixture at 65°C for 5 minutes. Precipitate the labeled RNA with ethanol.

5. References

1. Shinshi, H. *et al.*, (1976) *Biochemistry* **15**, 2185.
2. Shinshi, H. *et al.*, (1976) *FEBS Letters* **65**, 254.
3. Schaefer, B.C. (1995) *Anal. Biochem.* **227**, 255.
4. Efstratiadis, A. *et al.*, (1977) *Nucleic Acids Res.* **4**, 4165.

6. Related Products

The following products are also available:

Cat. #	Concentration	Quantity
APex™ Heat-Labile Alkaline Phosphatase		
AP49100	1 Reaction/µl	100 Reactions
T4 Polynucleotide Kinase		
P0503K	10 U/µl	3,000 Units
RNA 5′ Polyphosphatase		
RP8092H	20 U/µl	200 Units

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