

## Tobacco Acid Pyrophosphatase Efficiently Removes the 5'-Cap Structure From Eukaryotic mRNAs

The 5'-termini of many natural RNA molecules, including most eukaryotic RNAs, viral RNAs and many small nuclear RNAs, have a 5'-terminal methylated guanine nucleotide structure called a "cap". Tobacco Acid Pyrophosphatase (TAP) hydrolyzes the phosphoric acid anhydride bonds in the triphosphate bridge of the cap structure, releasing the cap nucleoside and generating a 5'-phosphorylated terminus on the RNA molecule (FIG 1). The resulting "decapped" 5'-phosphorylated terminus may be ligated to a 3'-hydroxylated terminus using T4 RNA Ligase (EPICENTRE Biotechnologies) or dephosphorylated using APex™ Heat-Labile Alkaline Phosphatase (EPICENTRE) for end labeling. TAP also digests the

triphosphate group at the 5'-end of prokaryotic transcripts, generating an RNA molecule with a 5'-phosphorylated terminus. TAP is function-tested in an RNA decapping assay,<sup>1</sup> and is free of detectable RNase activity.

### Applications include:

- Preparation of templates for rapid amplification of cDNA ends (RACE).<sup>2</sup>
- Ligation of oligoribonucleotides to TAP-treated cellular RNA for construction of full-length cDNA libraries.<sup>3</sup>
- Mapping of transcription sites for eukaryotic<sup>4</sup> and prokaryotic<sup>5</sup> transcripts.
- Radiolabeling of RNA<sup>6</sup> for use in sequencing or as a hybridization probe.

### References

1. (2004) EPICENTRE Forum 11(5), 23.
2. Schaefer, B.C. (1995) *Anal. Biochem.* 227(2), 255.
3. Oh, J.H. *et al.*, (2003) *Exp. Mol. Med.* 35(6), 586.
4. Li, W. *et al.*, (2003) *J. Biosci.* 28(6), 691.

5. Bensing, B.A. *et al.*, (1996) *Proc. Natl. Acad. Sci.* 93(15), 7794.
6. Efstratiadis, A. *et al.*, (1977) *Nucleic Acids Res.* 4(12), 4165.

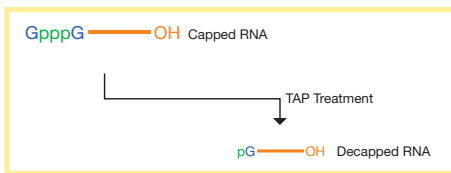


FIG 1. Tobacco Acid Pyrophosphatase removes the 5'-Cap structure from eukaryotic mRNAs generating a 5'-phosphorylated RNA.

[www.EpiBio.com/tap.asp](http://www.EpiBio.com/tap.asp)

### Tobacco Acid Pyrophosphatase

T19050	50 Units
T19100	100 Units
T19250	250 Units
T19500	500 Units

[www.EpiBio.com/apex.asp](http://www.EpiBio.com/apex.asp)

### APex™ Heat-Labile Alkaline Phosphatase

AP49010	10 Reactions
AP49050	50 Reactions
AP49100	100 Reactions

[www.EpiBio.com/t4\\_rna\\_ligase.asp](http://www.EpiBio.com/t4_rna_ligase.asp)

### T4 RNA Ligase

LR5010	5 U/μl 1,000 Units
LR5025	5 U/μl 2,500 Units
LR5050	5 U/μl 5,000 Units

Includes 10X Reaction Buffer and a 10 mM ATP Solution.

## Impressive Reproducibility of ArrayPure™ RNA Purifications

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Microarray technology offers an opportunity to assess the reproducibility of RNA preparations over the entire cellular transcriptome—the complete collection of all of a cell's mRNA molecules (transcripts). One possible approach is to convert two replicate preparations of RNA into microarray targets labeled with different fluorescent dyes, and hybridize them to the same array. The normalized fluorescence intensity of each spot is proportional to the abundance of a particular transcript in the RNA preparation. Therefore, a scatter plot displaying the Log<sub>2</sub> signal intensities for the two dyes provides information on how comparable the two RNA preparations are; the more similar they are, the more closely the plot approximates a straight line with a slope of 45°.

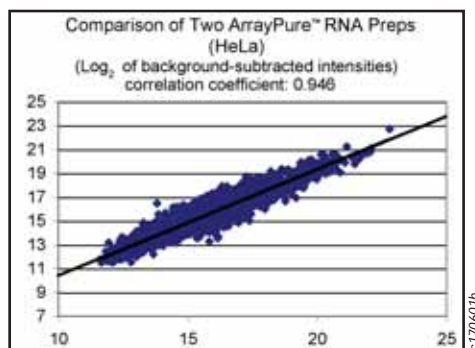


FIG 1. Scatter plot of microarray data indicates a high level of ArrayPure™ purification reproducibility. HeLa cell RNA was purified from two separate T25 tissue culture flasks, labeled with Cy™3 or Cy5, and hybridized to a microarray containing Operon Biotechnologies' Array-Ready Human Oligo Set™ (70-mer). Spotting, target labeling, and hybridizations were conducted by the University of Cincinnati Genomics and Microarray Laboratory.

FIG 1 shows such a scatter plot of microarray data from two HeLa cell RNA purifications prepared from two separate tissue culture flasks with a ten-fold scaled-up version of the ArrayPure™ Nano-scale RNA Purification Kit. A Pearson correlation coefficient of 0.946 indicates a high level of reproducibility of replicate ArrayPure™ RNA purifications.

[www.EpiBio.com/arraypure.asp](http://www.EpiBio.com/arraypure.asp)

### ArrayPure™ Nano-scale RNA Purification Kit

MPS04050	50 Purifications
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