

Tobacco Acid Pyrophosphatase (TAP) Functional Assay Ensures Decapped mRNA

Tobacco acid pyrophosphatase (TAP) removes the “cap” structure from the 5′-end of eukaryotic mRNAs,¹ or the 5′-triphosphate from unprocessed prokaryotic transcripts,² leaving a 5′-monophosphate. This prepares the RNA molecule for ligation to other acceptor molecules. For example, T4 RNA ligase has been used to ligate oligoribonucleotides to TAP-treated cellular RNAs, allowing construction of full-length cDNA libraries³ and accurate mapping of transcription initiation sites, for both eukaryotic⁴ and prokaryotic² transcripts.

TAP activity is routinely assayed, using ATP^{1,5} or *p*-nitrophenyl phosphate⁵ as the substrate, by measuring the liberation of phosphate or *p*-nitrophenol. Assays are also performed to ensure that TAP prepa-

rations are not contaminated with phosphatases, which could remove the monophosphate remaining on the 5′ end of the decapped RNA, or with RNases, which could degrade the RNA substrate. However, none of these assays directly determine that the enzyme specifically decaps an RNA substrate.

Functional TAP assay

Each lot of EPICENTRE’s TAP is tested in a functional assay, as outlined in Figure 1. A short, capped RNA transcript is synthesized using EPICENTRE’s AmpliCap™ SP6 High Yield Message Maker Kit and incubated in the presence or absence of TAP. Each reaction is divided into two and incubated either in the presence or absence of T4 RNA ligase.

The ligation reaction is assayed on an 8 M urea, 20% polyacrylamide gel in a Tris-borate-EDTA buffer and stained with SYBR® Gold. Figure 2 shows that only capped RNA that was treated with TAP and RNA Ligase produced ligation products, as indicated by a shift in the RNA band from the non-ligated position to the slower migrating ligated position.

Conclusion

EPICENTRE functionally assays our TAP to ensure the expected specificity on capped RNA molecules. Specific ligation products would not be observed if the TAP was inactive, contaminated with a phosphatase, which would remove the 5′ phosphate from the decapped RNA, or contaminated with ribonuclease, which would produce smaller non-ligatable degradation products containing 3′ phosphates.

References

1. Efstratiadis, A. *et al.* (1977) *Nucleic Acids Res.* **4**, 4165.
2. Bensing, B. A. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**, 7794.
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. Shinshi, H. *et al.* (1976) *Biochemistry* **15**, 2185

Figure 1. Schematic outline of EPICENTRE’s functional TAP assay.

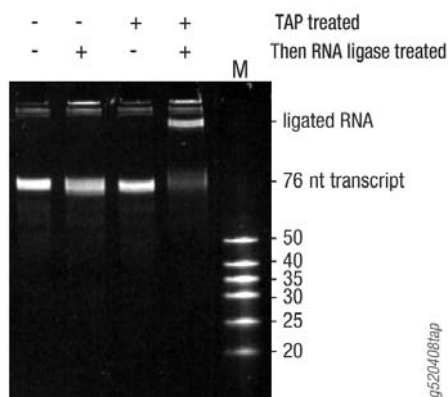
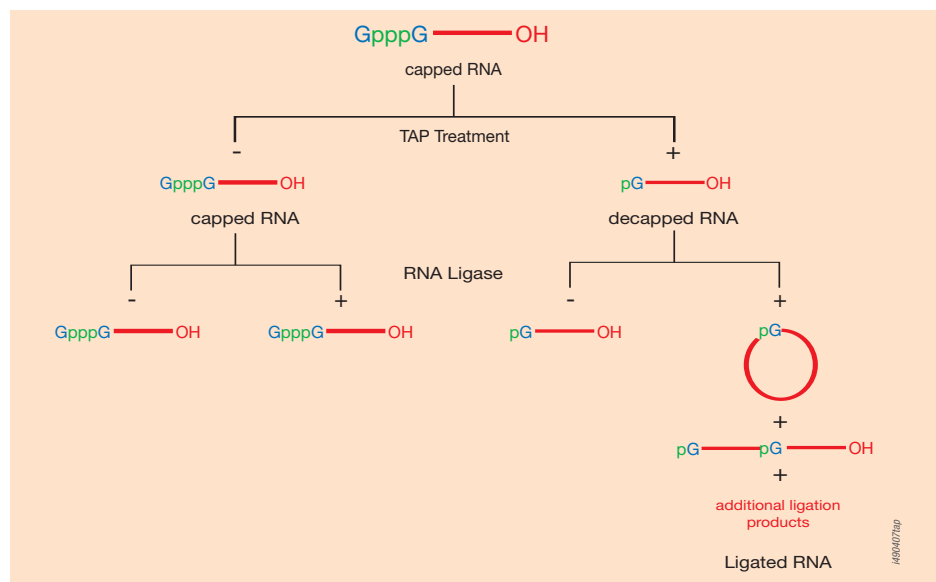


Figure 2. Lot # 750-30850 TAP treatment of capped RNA allows efficient self-ligation.

www.epicentre.com/tap.asp

Tobacco Acid Pyrophosphatase (TAP)

T19050	50 U
T19100	100 U
T19250	250 U
T19500	500 U
Includes 10X Reaction Buffer.	

AmpliCap™ SP6 High Yield Message Maker Kit

AC0706	25 Reactions
LR5010	5 U/μl 1,000 U
LR5025	5 U/μl 2,500 U
LR5050	5 U/μl 5,000 U
Includes 10X Reaction Buffer and a 10 mM ATP Solution.	

T4 RNA Ligase is also available in bulk. Please inquire.

SYBR® Gold is a registered trademark of Molecular Probes.