



## TSAP: A New Thermosensitive Alkaline Phosphatase

**ABSTRACT** Here we describe TSAP, a new thermosensitive alkaline phosphatase. TSAP is active in all Promega restriction enzyme buffers, including MULTI-CORE™ Buffer, as well as both GoTaq® Buffers. The robust phosphatase activity effectively dephosphorylates all DNA termini (5', 3' and blunt) in 15 minutes at 37°C. As TSAP is active in restriction enzyme buffers, using TSAP offers the potential for simultaneous restriction digestion/dephosphorylation. Promega TSAP is thoroughly tested to ensure functionality and physical purity.

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### INTRODUCTION

TSAP, Thermosensitive Alkaline Phosphatase (Cat.# M9910), catalyzes the removal of 5' phosphate groups from DNA, thus preventing the recircularization of linearized cloning vector DNA during ligation. It is effective on 3' overhangs, 5' overhangs, and blunt ends. The phosphatase is supplied at a concentration of 1MBU/μl, which is a sufficient quantity to dephosphorylate 1μg of plasmid (~3kb) in 15 minutes at 37°C. It is also useful for preparing DNA for 5' end-labeling by removing existing phosphate groups from the 5' end (T4 Polynucleotide Kinase, Cat.# M4101). TSAP is irreversibly inactivated by incubating at 74°C for 15 minutes. Therefore, a DNA cleanup step is not required before proceeding to a ligation reaction.

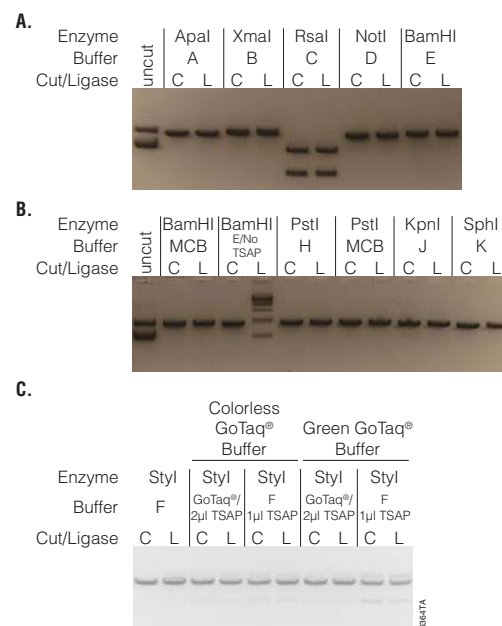
Most alkaline phosphatases have several disadvantages in a practical world. Many require a metal cofactor such as Zn<sup>2+</sup>, which renders them incompatible with common restriction enzyme buffers. In addition, *E. coli* alkaline phosphatase and calf intestinal alkaline phosphatase are not inactivated by heating and require a DNA purification step before ligation. This can be costly and laborious and can decrease yield. Finally, consecutive enzyme incubations, with resultant buffer additions, can result in added time, loss of DNA and increased chance for errors.

### SIMULTANEOUS DEPHOSPHORYLATION AND RESTRICTION DIGESTION

Active in Promega restriction buffers, TSAP will allow simultaneous restriction enzyme digestion and DNA dephosphorylation under the appropriate conditions (Figure 1). Using TSAP for cloning can minimize the time and manipulation (Figure 2).

**Robust activity**  
Effectively dephosphorylates all types of DNA termini in 15 minutes.

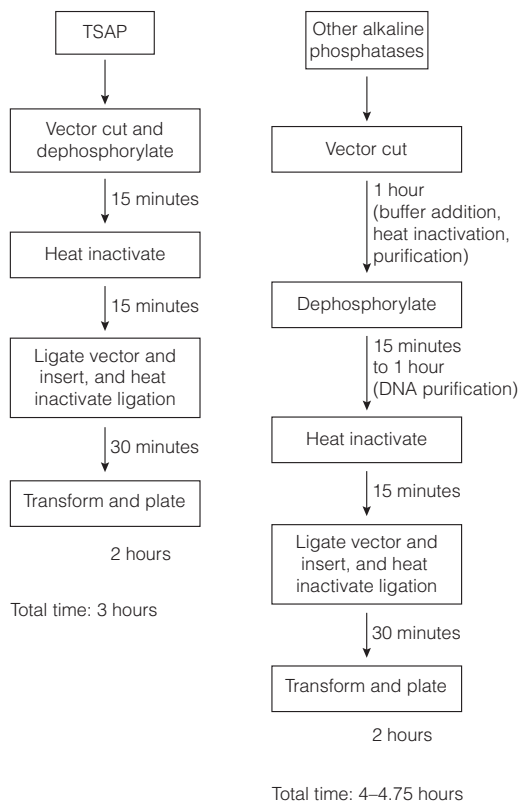
**Flexible and efficient**  
Compatible with all Promega restriction enzyme buffers, TSAP is active while the enzyme is cutting.



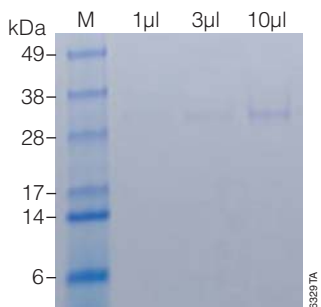
**Figure 1. Phosphatase activity as demonstrated by ligation inhibition.** Two micrograms of pGEM<sup>®</sup>-3Zf(+), pGEM<sup>®</sup>-5Zf(+), or pBR322 plasmid DNA were incubated with 2 units of TSAP and 15 units of the indicated restriction enzyme and Promega buffer. Following 15 minutes at 37°C, the TSAP/restriction enzyme cocktail was heat inactivated by incubating for 15 minutes at 74°C. The resulting DNA was split into two aliquots. One aliquot was saved (C=cut) and one aliquot was ligated using 20 units of ligase and 1X LigaFast™ Buffer for 15 minutes at 25°C (L=ligated). The ligation reaction was heat inactivated by incubating for 15 minutes at 65°C. **Panel A.** pGEM<sup>®</sup>-5Zf(+). Plasmid DNA (Apal and NotI) and pGEM<sup>®</sup>-3Zf(+). Plasmid DNA (Xmal, RsaI and BamHI). **Panel B.** pGEM<sup>®</sup>-3Zf(+). Plasmid DNA. Two units of TSAP/μg of DNA was used for StylI/buffer F and either Green GoTaq<sup>®</sup> Buffer or Colorless GoTaq<sup>®</sup> Buffer, as TSAP is slightly less active at higher pH and Mg<sup>2+</sup> levels.

**UNITS DEFINED BY FUNCTION**

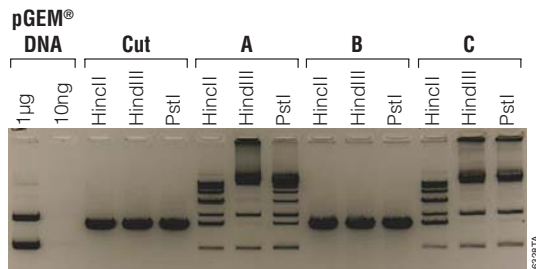
Promega defines unit activity on a functional basis. One unit of TSAP will dephosphorylate 1µg of DNA (~3kb plasmid with a 3', 5' or blunt end) in 15 minutes at 37°C. TSAP is heat inactivated with a 15-minute incubation at 74°C. While many manufacturers use *p*-nitrophenyl phosphate (PNPP) as a substrate in their unit definition, it should be noted that TSAP does not exhibit strong activity on PNPP. However, reduced PNPP activity does not affect DNA dephosphorylation.



**Figure 2. Comparison of TSAP protocol to standard alkaline phosphatase protocol.**



**Figure 3. Polyacrylamide gel analysis of TSAP purity.** One, 3 and 10µl (MBU) of two different lots of TSAP were separated on a 4–12% NuPage® polyacrylamide gel (Invitrogen), visualized using SimplyBlue™ stain (Invitrogen) and captured with a digital camera. Lane M, SeeBlue® prestained protein standard (Invitrogen).



**Figure 4. Gel analysis of TSAP activity.** Functional purity is gauged by the absence of altered DNA ends. pGEM®-3Zf(+) Vector DNA was digested with either HincII (blunt end), HindIII (5' end) or PstI (3' end). The digested DNA was then subjected to different treatments. Treatment A: Cut DNA (2µg) was ligated using 1X ligase buffer (Cat.# C1263), 8u LC Ligase (Cat.# M1801) and incubated at 4°C for 16 hours. Treatment B: Cut DNA (2µg) was incubated with TSAP for 1 hour at 37°C, purified with the Wizard® Plus Minipreps DNA Purification System (Cat.# A7100) and then ligated. Treatment C: Cut DNA (4µg) was incubated with TSAP, treated with T4 Polynucleotide Kinase using 1X PNK Buffer (Cat.# C1313) with added ATP, 40 units/4µg T4 PNK (Cat.# M4101) and incubated at 37°C for 10 minutes, and then ligated. Following these treatments, aliquots (1µg) were separated on a 1% agarose gel, visualized by ethidium bromide staining and captured with a digital camera.

**PHYSICAL AND FUNCTIONAL PURITY**

TSAP is manufactured in an ISO-certified facility, ensuring consistent quality as evidenced by the physical and functional purity of the TSAP enzyme (Figures 3 and 4).

**SUMMARY**

Promega TSAP offers increased efficiency, effectively dephosphorylating all types of DNA termini in 15 minutes. TSAP is compatible with all Promega restriction enzyme buffers, offering the potential of simultaneous restriction enzyme digestion and DNA dephosphorylation. Because TSAP can be irreversibly heat inactivated, a DNA purification step is not required prior to ligation. Finally, TSAP comes with the assurance in quality that arises from extensive testing using methods that are sensitive to contaminating activities.

**ACKNOWLEDGMENTS**

We wish to acknowledge Ray Williams and Erik Gulliksen for their continual support and sound advice.

**ORDERING INFORMATION**

Product	Size	Cat.#
TSAP Thermosensitive Alkaline Phosphatase	100 units	M9910

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