Certificate of Analysis

TSAP Thermosensitive Alkaline Phosphatase, Blue/White Cloning Qualified:

Part# 9PIM991

Revised 03/18

Cat.#	Size	Supplied With: MULTI-CORE™ 10X Buffer
M9910	100 units (M991A)	250µl (R999A)

Description: TSAP Thermosensitive Alkaline Phosphatase catalyzes the removal of 5' phosphate groups from DNA, thus preventing the recircularization and religation of linearized cloning vector DNA during ligation. It is effective on 3' overhangs, 5' overhangs and blunt ends. It is also useful for preparing DNA for 5' end-labeling by removing existing phosphate groups from the 5' end.

TSAP is irreversibly inactivated by heating at 74°C for 15 minutes. Therefore, a DNA cleanup step is not required before proceeding to a ligation reaction. TSAP is fully active in all Promega restriction enzyme reaction buffers under the conditions listed on the back of this document, facilitating a streamlined restriction digestion, dephosphorylation and ligation reaction. MULTI-CORE™ 10X Buffer is provided with the enzyme for your convenience.

Enzyme Concentration: 1 Molecular Biology Unit per microliter (1MBU/µl).

Enzyme Storage Buffer: 10mM Tris-HCl (pH 7.5 at 25°C), 100mM NaCl and 50% glycerol.

MULTI-CORE™ 10X Buffer (R999A): 250mM Tris-acetate (pH 7.8 at 25°C), 1M potassium acetate, 100mM magnesium acetate and 10mM DTT.

Source: Bacteria.

Unit Definition: One Molecular Biology Unit (MBU) is defined as the amount of enzyme required to dephosphorylate 1µg of linearized pGEM®-3Zf(+) DNA in 15 minutes at 37°C in any 1X Promega Restriction Enzyme Buffer, except Buffer F.

Storage Temperature: See the Product Information Label for storage recommendations and expiration date.

Usage Note for MULTI-CORE™ 10X Buffer: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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Quality Control Assays

Blue/White Assay: TSAP is tested for its suitability for cloning applications under the assay conditions described. Less than 2% white colonies are produced when the starting material is DNA with a 5' or 3' overhang, and less than 5% white colonies are produced when the starting material is DNA with blunt ends. **Blue/White Assay Conditions:** pGEM®-3Zf(+) DNA is digested with representative restriction enzymes (leaving 5'-termini, 3'-termini or blunt ends), and 15µg of each digested DNA is incubated with 30 units of TSAP for 1 hour at 37°C. The DNA is purified using the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281), and the purified DNA is then rephosphorylated using T4 Polynucleotide Kinase (Cat.# M4101), ligated, and transformed into JM109 Competent Cells (Cat.# L2001). The cells are plated and incubated overnight at 37°C on LB plates containing ampicillin, IPTG and X-gal. The next day a minimum of 200 colonies are counted for each sample, and the % white colonies produced are calculated.

Functional Assay: To test the ability of TSAP to effectively dephosphorylate DNA, TSAP is used to dephosphorylate pGEM®-3Zf(+) DNA that has been digested with representative restriction enzymes (leaving 5'-termini, 3'-termini or blunt ends). The dephosphorylated DNA is ligated overnight at 4°C and visualized by ethidium bromide staining following agarose gel electrophoresis. Under these conditions, the DNA predominantly produces a single band of equivalent size to pGEM®-3Zf(+) DNA that has been cut but not ligated.

Nuclease Assays: No contaminating endonuclease, exonuclease or RNase activity detected.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

1. Streamlined Restriction Digestion, Dephosphorylation and Ligation

Since TSAP is active in all Promega restriction enzyme buffers, the vector DNA can easily be restriction digested and dephosphorylated at the same time.

1. As a general guideline, for reactions containing up to 1µg of DNA, add 15 units of restriction enzyme and the amount of TSAP listed below to the vector DNA in a total reaction volume of 20–50µl. Set up the reaction in the appropriate 1X Promega restriction enzyme reaction buffer.

Reaction Buffer	Amount of TSAP for Reactions Containing ≤1µg DNA
Promega 10X Reaction Buffers A–L (except F)	1µl
Promega 10X Reaction Buffer F	2µl
MULTI-CORE™ 10X Buffer	1µl

2. Incubate the reaction at 37°C for 15 minutes. This is a sufficient amount of time to digest and dephosphorylate all vector DNA overhang types (3', 5' or blunt).
3. Heat-inactivate the TSAP and the restriction enzyme by incubating the reaction at 74°C for 15 minutes.

   Note: Not all restriction enzymes can be heat-inactivated. If the restriction enzyme cannot be heat-inactivated, clean up the digest using the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).
4. Briefly centrifuge the reaction, and use approximately 40ng of dephosphorylated vector in a ligation reaction containing DNA insert, 1X Rapid Ligation Buffer, and 2µl (6 units) of T4 DNA Ligase (LigaFast™ Rapid DNA Ligation Sytem, Cat.# M8221). Incubate ligation reactions containing vector with 5' or 3' overhangs at 25°C for 5 minutes. Incubate ligation reactions containing vector with blunt ends at 25°C for 15 minutes.

   Note: Optimal vector-to-insert ratios may need to be determined. We recommend using a 1:2 molar ratio of vector to insert DNA as a starting point. (See the *LigaFast™ Rapid DNA Ligation System Product Information Sheet* #9PIM822 available at: **www.promega.com/protocols/** for additional information).
5. Transform the ligated material directly into *E. coli* competent cells following the recommended transformation protocol provided with the cells.

2. Sequential Digestion and Dephosphorylation Reaction

Alternatively, one can digest the vector DNA before proceeding to the dephosphorylation reaction.

1a. Set up the restriction digest according to the directions provided with the restriction enzyme in a reaction volume of 20–50µl. When the digest is complete, heat-inactivate the restriction enzyme according to the directions provided with the restriction enzyme.

Note: Not all restriction enzymes can be heat-inactivated. If the restriction enzyme cannot be heat-inactivated, purify the digest using the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281), and add MULTI-CORE™ 10X Buffer to a final concentration of 1X before proceeding to Step 1b.

1b. Add 1µl of TSAP for reactions containing up to 1µg of digested DNA. **Do not add extra buffer.**

Note: If Restriction Enzyme Buffer F was used in the restriction digest and the digest was **not** purified with the Wizard® SV Gel and PCR Clean-Up System, use 2µl of TSAP

2. Incubate the reaction at 37°C for 15 minutes. This is a sufficient amount of time to dephosphorylate all vector DNA overhang types (3', 5' or blunt).
3. Proceed to Step 3 of "Streamlined Restriction Digestion, Dephosphorylation and Ligation".

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