EURx® MOLECULAR BIOLOGY PRODUCTS

TspGWI

5'-A C G G A $(N)_{11}$-3'
3'-T G C C T $(N)_9$-5'

Cat. No.	size
E2501-01	50 units
E2501-02	250 units

Reaction Temperature: 70°C

Inactivation Temperature (20 min): –

Prototype: TspGWI

Source: *Thermus species GW*
Purified from *E.coli* strain that carries the cloned tspGWRI gene from *Thermus sp. GW*.

Package Contents:

- **TspGWI**
- **10 x Reaction Buffer TspGWI**

Storage Conditions: Store at -20°C.

Prepare and store buffer aliquots at -70°C.

DNA Methylation:

No Inhibition: dam, dcm, EcoKI,

Potential Inhibition: CpG

References:

1. *Żylicz-Stachula, A., Harasimowicz-Słowińska, R. Sobolewski, I. and Skowron, P., (2002). Nucleic Acids Research 30, 7 e 33.*

Standard Reaction Protocol (for 50 µl volume):

Mix the following reaction components:

1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)

5 µl 10 x Buffer TspGWI

2 U TspGWI (use 1 U per µg DNA, < 10% React. Volume!)

Tips: Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.

add sterile H_2O to 50 µl final volume

Incubate for more than2 h at 70°C

Stop reaction by alternatively

(a) Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM *or*

(b) Heat Inactivation

(not applicable for this enzyme) *or*

(c) Spin Column DNA Purification

(e.g. EURx PCR/DNA Clean-Up Kit, Cat.No. E3520) *or*

(d) Gel Electrophoresis and Single Band Excision

(e.g. EURx Agarose-Out DNA Kit, Cat.No. E3540) *or*

(e) Phenol-Chloroform Extraction or Ethanol Precipitation.

Note 1: It is required to purify DNA before digestion. We recommend PCR/DNA Clean-Up Purification Kit or Agarose-Out DNA Purification Kit.

Note 2: It is not recommended to use more than 2 units per 50 µl reaction. Digestion should be performed for over 2 hr. As TspGWI binds DNA very tightly, excess amount of TspGWI added can retard DNA migration on a gel, even in the presence of denaturing agents.

Note 3: Restriction endonuclease TspGWI is very highly stimulated by the presence of two restriction sites in opposite orientation. Both the distance between recognition sequences and their immediate neighborhood also affects the cleavage effectiveness. Single site substrates are cleaved slowly.

Unit Definition:

One unit is the amount of enzyme required to digest 1 µg of pBR322 DNA to obtain stable digestion pattern in 1 hr. Total reaction volume is 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x TspGWI Buffer: 10 mM Tris-HCl (pH 8.5 at 25°C), 1 mM dithiothreitol, 10 mM $MgCl_2$ +enhancers (1).

Avoid multiple cycles of freezing/thawing of the stock reaction buffer (no more than 3 times). Thawing should be performed at temperatures not exceeding 10°C. Recommended procedure is to divide the provided reaction buffer into smaller portions and preserve them at –70°C for long-term. Temperature of –20°C should be used only for short-term storage.

Storage Buffer:

20 mM Tris-HCl (pH 8.3 at 25°C), 25 mM $(NH_4)_2SO_4$, 25 mM KCl, 0.5 mM EDTA, 0.5 mM dithiothreitol, 0.02% Tergitol™ TMN, 0.02% Tween™20, 0.02% Igepal, 50% (v/v) glycerol.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as nonspecific single- and double-stranded DNase activities. Ligation / recut assay performed successfully.

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