TspDTI

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

Cat. No.	Size
E2502-01	50 units
E2502-02	250 units

Reaction Temperature: 70°C

Inactivation Temperature (20 min): --

Prototype: TspDTI

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

Package Contents:

- **TspDTI**
- **10x Reaction Buffer TspDTI**

Storage Conditions: Store at -20°C
Prepare and store buffer aliquots at -70°C.

Double Digestion – Buffer Compatibility:

Buffer	% Rel. Activity
Low	NR***
Medium	NR***
High	NR***
Acet	NR***

*** NR - buffer is not recommended, use 1 x buffer TspDTI.

Recommended Buffer: TspDTI
(or compatible third party buffers)

DNA Methylation:
No inhibition: dam, dcm, EcoKI, CpG

Standard Reaction Protocol:

Mix the following reaction components:
1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
3 µl 10x Buffer TspDTI
1-2 U TspDTI (use 1 U / 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.
@ 30 µl H_2O, nuclease free

Incubate for 3 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 CleanUp Kit, Cat.No. E3520) *or*
(d) Gel Electrophoresis and Single Band Excision
(e.g. EURx AgaroseOut 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 30 µl reaction. It is strongly suggested to perform digestion for over 1 hr.
Note 3: To avoid DNA shift during electrophoresis caused by strong protein-DNA interaction, it is recommended to terminate reaction by addition of reaction stop solution (containing denaturing reagent, i.e. 0,2% SDS) followed by 20 minutes heat inactivation in 89°C.

Unit Definition:

One unit is the amount of enzyme required to digest 1 µg of pUC19 DNA to obtain stable digestion pattern in 1 hr in a total reaction volume of 30 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x TspDTI Buffer: 10 mM Tris-HCl (pH 8.5 at 25°C), 10 mM $MgCl_2$, 1 mM dithiothreitol +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 % Triton™X-100, 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 non-specific single- and double-stranded DNase activities.