



CERTIFICATE OF ANALYSIS

TscAI (TspRI)

#ER2101 1000 u

Lot: Expiry Date:

C

5'... N N C A G T G N N↓...3'

3'...↑N N G T C A C N N ...5'

G

Concentration: 10 u/μl
Source: *Thermus scotoductus* RFL5
Supplied with: 1 ml of 10X Buffer Tango™

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer Tango™ (for 100% TscAI digestion)
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

Incubation temperature

65°C*.

Unit Definition

One unit is defined as the amount of TscAI required to digest 1 μg of lambda DNA in 1 hour at 65°C in 50 μl of recommended reaction buffer.

Dilution

Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

Double Digests

Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to the Fermentas Catalog or go to www.fermentas.com/doubledigest to choose the best buffer for your experiments.

* Incubation at 37°C results in less than 5% activity.

Storage Buffer

TscAI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 μ l
10X Buffer Tango™	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
TscAI	0.5-2 μ l**
- Mix gently and spin down for a few seconds.
- Incubate at 65°C for 1-16 hours**.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 μ l (~0.1-0.5 μ g of DNA)
nuclease-free water	18 μ l
10X Buffer Tango™	2 μ l
TscAI	1-2 μ l**
- Mix gently and spin down for a few seconds.
- Incubate at 65°C for 1-16 hours**.

** See Note on back page.

Thermal Inactivation

TscAI is not inactivated by incubation at 80°C for 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
 - stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
 - extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
 - dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
 - check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **QUALITY CONTROL ASSAY DATA**
see back page

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

B	G	O	R	Tango™	2X Tango™
50-100	50-100	20-50	20-50	100	20-50

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: never overlaps – no effect.

EcoKI: may overlap – effect not determined.

EcoBI: may overlap – effect not determined.

Stability during Prolonged Incubation

A minimum of 0.2 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 65°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
119	6	12	10	10	10	8

Note

- A large excess of TscAI (7.5 u/µg DNA x 16 hours) may result in star activity.
- TscAI produces DNA fragments with a 9-base 3'-extension. To avoid atypical DNA band patterns, use 6X DNA Loading Dye & SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of 0.1% SDS (final concentration) at 65°C for 10 min prior to electrophoresis.

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after an 80-fold overdigestion with TscAI (5 u/µg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with TscAI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 1.12 µM. More than 95% of these sites can be recut.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of TscAI for 4 hours.

Quality authorized by:



Jurgita Zilinskiene

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product