Thermo SCIENTIFIC

PRODUCT INFORMATION

TscAI (TspRI)

#ER2101 1000 U

Lot: ___ **Expiry Date: _**

5'... **N N C A S T G N N**↓...3'
3'...↑**N N G T S A C N N** ...5'

Concentration: 10 U/µL
Source: *Thermus scotoductus* RFL5
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (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 provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to www.thermoscientific.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 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
TscAI	0.5-2 µL**

- 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 µL (~0.1-0.5 µg of DNA)
nuclease-free water	18 µL
10X Buffer Tango	2 µL
TscAI	1-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 65°C for 1-16 hours**.

** *See* Overdigestion Assay 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 **CERTIFICATE OF ANALYSIS** *see* back page

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific 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

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.

CERTIFICATE OF ANALYSIS

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 and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

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