Thermo SCIENTIFIC

PRODUCT INFORMATION

I-SceI

#ER1771 250 U

Lot: ___ Expiry Date: _

5'...**T A G G G A T A A↓C A G G G T A A T** ...3'

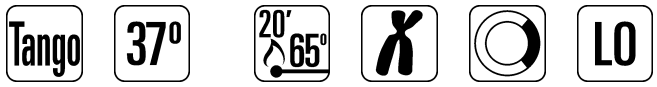
3'...**A T C C C↑T A T T G T C C C A T T A** ...5'

Concentration: 10 U/µL

Source: *E.coli* that carries the cloned *I-SceI* mitochondrial gene from *Sacharomyces cerevisiae*

Supplied with: 1 mL of 10X Buffer Tango
1 mL of 10X Buffer Tango without Mg-acetate
1 mL of 100 mM Mg-acetate

Store at -20°C



BSA included

www.thermoscientific.com/onebio

Description

I-SceI is a site-specific homing endonuclease encoded by a mitochondrial intron of *Saccharomyces cerevisiae*.

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% I-SceI digestion)

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of I-SceI required to digest 1 µg of pUC-I-SceI DNA in 1 hour at 37°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.

Storage Buffer

I-SceI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 500 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 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.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
I-SceI	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°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
I-SceI	1-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

I-SceI is inactivated by incubation at 65°C for 20 min.

Digestion of agarose-embedded DNA

For efficient digestion of an agarose embedded DNA, agarose plugs should be incubated on ice for 2 hours in 50-100 µL of 1X Tango buffer **without Mg-acetate** (because I-SceI is unstable in the presence of Mg^{2+} ions) in the presence of I-SceI enzyme (20 units/plug). This step allows I-SceI to reach and bind its target through the agarose. The reaction is subsequently started by the addition of Mg-acetate at a final concentration of 10 mM and the incubation of plugs is carried out at 37°C for 1 hour.

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	50-100	50-100	**100**	50-100

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of 1 µg of DNA in 16 hours at 37°C.

Note

- Homing endonucleases do not have stringently defined recognition sequences. They can tolerate minor sequence changes, which only partially affect the cleavage reaction.
- I-SceI may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band patterns, use the 6X DNA Loading Dye&SDS Solution (R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with I-SceI (10 U/µg 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 I-SceI for 4 hours.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

Quality authorized by:  Jurgita Zilinskiene

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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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.