

**PRODUCT INFORMATION**

# FspAI

**#ER1661** 100 U

**Lot:** \_\_\_\_\_ **Expiry Date:** \_\_\_\_\_

5'...**R** T G C↓G C A Y...3'

3'...Y A C G↑C G T R...5'

Concentration: 5 U/μL

Source: *Flexibacter species* Tv-m21K

Supplied with: 1 mL of 10X Buffer 0

1 mL of 10X Buffer Tango

**Store at -20°C**



**0**

**37°**

**CG**



**LO**

BSA included

[www.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio)

## RECOMMENDATIONS

**1X Buffer 0** (for 100% FspAI digestion)

50 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 100 mM NaCl, 0.1 mg/mL BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of FspAI required to digest 1 μg of lambda DNA-Psp1406I fragments 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.

**Double Digests**

Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango<sup>TM</sup> Buffer. Please refer to

[www.thermoscientific.com/doubledigest](http://www.thermoscientific.com/doubledigest) to choose the best buffer for your experiments.

1X Tango Buffer: 33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

## Storage Buffer

FspAI is supplied in: 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.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer O	2 µL
DNA (0.5-1 µg/µL)	1 µL
FspAI	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 O	2 µL
FspAI	1-2 µL*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

\* See Overdigestion Assay.

### Thermal Inactivation

FspAI is inactivated by incubation at 65°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
0-20	0-20	100	50-100	0-20	50-100

### Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: completely overlaps – blocked.

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 37°C.

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
2	0	2	0	0	0	0

For **CERTIFICATE OF ANALYSIS** see back page

# CERTIFICATE OF ANALYSIS

## Overdigestion Assay

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

## Ligation and Recleavage (L/R) Assay

The ligation and reclavage 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 FspAI 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

## **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](http://www.thermoscientific.com/onebio) for Material Safety Data Sheet of the product.

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