Thermo SCIENTIFIC

PRODUCT INFORMATION

FaqI (BsmFI)

#ER1811 100 U

Lot: ___ **Expiry Date: _**

5'...**G G G A C(N)$_{10}$**↓...3'
3'...**C C C T G(N)$_{14}$**↑...5'

Concentration:	2 U/µL
Source:	*Flavobacterium aquatile* RFL1
Supplied with:	1 mL of 10X Buffer Tango
	0.1 mL of 50X SAM Solution (2.5 mM)

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

[1X Thermo Scientific Tango Buffer] + SAM*

(for 100% FaqI digestion)
[33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA] + 0.05 mM S-adenosylmethionine (SAM).

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of FaqI at which no change in the fragmentation pattern is observed with further increase of enzyme. 1 µg of lambda DNA is incubated with the enzyme for 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

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.

***** FaqI requires only Mg^{2+} for its activity, but is stimulated by S-adenosylmethionine. 0.05 mM S-adenosylmethionine gives more than a 2-fold increase in FaqI activity. Still, a complete cleavage of the some substrates with FaqI is difficult to achieve.

Storage Buffer

FaqI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 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
50X SAM	0.4 µL
FaqI	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
50X SAM	0.6 µL
FaqI	1-2 µL

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

Thermal Inactivation

FaqI is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 1 unit of the enzyme is required for 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
38	2	4	0	0	0/1	2

For **CERTIFICATE OF ANALYSIS** *see* back page

Note

- For cleavage with FaqI at least two copies of its recognition sequence are required.
- FaqI 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 64-fold overdigestion with FaqI (4 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 5 units of FaqI 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 for Material Safety Data Sheet of the product.