

## SfiI RS

5'-GGCCNNNNNGGCC-3'  
3'-CCGGNNNNNCCGG-5'

Cat. No.	size
E2381-01	2000 units
E2381-02	10000 units

**Reaction Temperature:** 37°C

**Inactivation Temperature (20 min):** –

**Prototype:** SfiI

**Source:** Recombinant. Purified from *E. coli* strain carrying the cloned SfiI gene from *Streptomyces fimbriatus*

**Package Contents:**

- SfiI RS
- 10 x ONE Buffer
- BSA [100x]  
Added as separate component to prevent reaction buffer precipitation.
- Dilution Buffer SfiI RS  
Added for enzymes exceeding 10 U/μl in concentration. High protein concentration warrants optimal stability during prolonged storage. Use dilution buffer to prepare short term working stocks (5-10 U/μl, non-freezing at -20°C).

**Storage Conditions:** Store at -20°C.

**Double Digestion – Buffer Compatibility:**

ONE Buffer is compatible with most EURx restriction enzymes.

**Note:** SfiI requires two copies of its recognition sequence for cleavage to occur.

**Restriction Enzyme Buffer Compatibility:**

Both, enzyme and buffers are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

**DNA Methylation:**

No Inhibition: dam, EcoKI

Potential Inhibition: dcm, CpG

### Standard Reaction Protocol (for 50 μl volume):

**Mix** the following reaction components:

- 1-2 μg pure DNA or 10 μl PCR product (≈0.1-2 μg DNA)
- 5 μl 10 x ONE Buffer
- 0.5 μl BSA [100x]
- 1-2 U SfiI RS (use 1 U per μg DNA, < 10% React. Volume!)

*Tips:* Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex. High (excess) amounts of enzyme can greatly speed up the reaction.

add sterile H<sub>2</sub>O to 50 μl final volume

**Incubate** for 1 h at 37°C

To obtain complete digestion of high molecular weight DNA, (e.g. plant genomic DNA), add excess amounts of enzyme and prolong the incubation time.

**Stop** reaction by alternatively

- (a) Addition of 2.1 μl EDTA pH 8.0 [0.5 M], final 20 mM or
- (b) Heat Inactivation  
(not applicable for this enzyme) or
- (c) Spin Column DNA Purification  
(e.g. EURx PCR/DNA Clean-Up Kit, Cat.No. E3520) or
- (d) Gel Electrophoresis and Single Band Excision  
(e.g. EURx Agarose-Out DNA Kit, Cat.No. E3540) or
- (e) Phenol-Chloroform Extraction or Ethanol Precipitation.

### Non-optimal buffer conditions:

To compensate for the lack of enzyme activity, increase the amount of enzyme and/ or reaction time accordingly. The following values may serve as orientation:

1. *Enzyme amount:* Instead of 1 U enzyme, use ~4 U of enzyme in buffers providing 25% rel. activity, ~2 U in 50%, ~1.5 U in 75% or ~1 U in 100%, respectively.
2. *Reaction time:* Increase by ~1.3-fold (75% rel. activity), ~2-fold (50%) or ~4-fold (25%).

### Unit Definition:

One unit is the amount of enzyme required to complete 98% digestion of 1 μg of pXba in 1 hr. Total reaction volume is 50 μl. Enzyme activity was determined in the recommended reaction buffer.

### Reaction Buffer:

1 x ONE Buffer

To be supplemented with 100 μg/ml bovine serum albumin.

### Storage Buffer:

5 mM K/PO<sub>4</sub> (pH 7.4 at 4°C), 300 mM NaCl, 0.1 mM EDTA, 10mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 0.15% (v/v) Tergitol™ TMN, 100 μg/ml bovine serum albumin and 50% (v/v) glycerol.

### Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as nonspecific single- and double-stranded DNase activities.