



FokI

Fok I

Restriction Endonuclease

Recognition Sequence:



Cat. No.	Size
E2170-01	500 units
E2170-02	2 500 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: FokI

Source: *Flavobacterium okeanokoites*

Package Contents:

- FokI
- 10x Reaction Buffer ONE
- BSA [100x]
 - Added as separate component to prevent reaction buffer precipitation.
- Dilution Buffer # 2
 - Added only for enzymes exceeding 10 U/μl in concentration. Use dilution buffer to dilute working stocks of enzyme to a customary concentration of 5 to 10 U/μl. Diluted enzyme stocks will not freeze during storage at -20°C.

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

ONE Buffer is compatible with most EURx restriction enzymes.

DNA Methylation:

No inhibition: dam, EcoKI
Potential inhibition: dcm, CpG

Standard Reaction Protocol:

Mix the following reaction components:

- 1-2 μg pure DNA or 10 μl PCR product (= ~0.1-2 μg DNA)
- 5 μl 10x Buffer ONE
- 1 μl BSA [100x]

1 U FokI (use 1 U / 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.

@ 50 μl H₂O, DNA and DNase free

Incubate for 1 h at 37°C

Stop reaction by alternatively

- (a) Addition of 2.1 μl EDTA pH 8.0 [0.5 M], final 20 mM *or*
- (b) Heat Inactivation
 - 20 min at 65°C *or*
- (c) Spin Column DNA Purification
 - (e.g. EURx PCR/DNA CleanUp Kit, Cat.No. E3520) *or*
- (d) Gel Electrophoresis and Single Band Excision
 - (e.g. EURx AgaroseOut DNA Kit, Cat.No. E3540) *or*
- (e) Phenol-Chloroform Extraction *or* Ethanol Precipitation.

Important Note:

It is not recommended

- to use more than one unit FokI per 1 μg of DNA
- to incubate for more than 2 hours.

Unit Definition:

One unit is the amount of enzyme required to completely digest 1 μg of Lambda DNA in 1 hr in a total reaction volume of 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.

Reaction 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.

Storage Buffer:

10 mM Tris-HCl (pH 7.4 at 25°C), 50 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.1 % Tween 20, 200 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.