FokI

5'-G G A T G $(N)_9$-3'
3'-C C T A C $(N)_{13}$–5'

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 Medium**
- **BSA [100x]**
  Added as separate component to prevent reaction buffer precipitation.
- **Dilution Buffer**
  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:

Buffer	% Rel. Activity
Low	NR***
Medium	**100**
High	NR***
Acet	NR***

*** NR - Not recommended.

Recommended Buffer: Medium

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 Medium
1.0 µl BSA [100x]
1-2 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 H_2O, nuclease 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 1 unit FokI per 1 µg of DNA and 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 Medium Buffer: 10 mM Tris-HCl (pH 7.5 at 37°C), 10 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol, 100 µg/ml bovine serum albumin.
To be supplemented with 200 µg/ml bovine serum albumin.

Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 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 non-specific single- and double-stranded DNase activities.