EURx® MOLECULAR BIOLOGY PRODUCTS

Sau3AI

5'-G A T C-3'
3'-C T A G-5'

Cat. No.	size
E2375-01	200 units
E2375-02	1000 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: MboI

Source: Recombinant. Purified from an *E.coli* strain carrying the cloned Sau3AI gene from *Staphylococcus aureus 3A*.

Package Contents:

- **Sau3AI**
- **10 x Reaction Buffer Sau3AI**
- **BSA with detergent [100x]**
  Added as separate component to prevent reaction buffer precipitation.
- **Dilution Buffer # Sau3AI**
  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.

DNA Methylation:

No Inhibition: dam, dcm, EcoKI

Potential Inhibition: 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 Buffer Sau3AI

0.5 µl BSA with detergent [100x]

1-2 U Sau3AI (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_2O 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

20 min at 65°C *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 completely digest 1 µg of unmethylated Lambda DNA in 1 hr. Total reaction volume is 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x Sau3AI Buffer

To be supplemented with 100 µg/ml bovine serum albumin and 0.025% detergent.

Note 1: Cleaves *Dam* methylated DNA in spite of sequence overlap.

Note 2: Adding 0.025% detergent and BSA to reaction is required for optimal digestion.

Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Tergitol™ TMN, 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.

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