# Sau3AI

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

| Cat. No. | Size |
|---|---|
| E2375-01 | 200 units |
| E2375-02 | 1 000 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**
- **5x Reaction Buffer Sau3AI**
- **BSA with Triton™X-100 [100x]**
  Added as separate component to prevent reaction buffer precipitation.

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

**Recommended Buffer: Sau3AI**

**DNA Methylation:**
No inhibition: dam, dcm, EcoKI
Potential inhibition: CpG

**Standard Reaction Protocol:**

***Mix*** the following reaction components:
1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
10 µl 5x Buffer Sau3AI
0.5 µl BSA with Triton™X-100[100x]
1-2 U Sau3AI (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. High (excess) amounts of enzyme can greatly speed up the reaction.
@ 50 µl $H_2O$, nuclease free

***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 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.

**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 in a total reaction volume of 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% Triton™X-100.

**Note 1: Cleaves *Dam* methylated DNA in spite of sequence overlap.**
**Note 2: Adding 0.025% Triton™X-100 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% Triton™X-100, 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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