# Sau3AI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R0169S 095120713071

## R0169S

NEB1 BSA 37° Yes

**200 units** **Lot: 0951207** **Exp: 7/13**
**RECOMBINANT** **4,000 U/ml** **Store at –20°C**

**Recognition Site:**

5′. . .▼GATC. . . 3′
3′. . . CTAG▲. . . 5′

**Source:** An *E. coli* strain that carries the cloned Sau3AI gene from *Staphylococcus aureus* 3A (J.S. Sussenbach)

**Now Recombinant**

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X NEBuffer 1, 100X BSA.

**Reaction Conditions:** 1X NEBuffer 1, supplemented with 100 µg/ml BSA. Incubate at 37°C.

**1X NEBuffer 1:**
10 mM Bis Tris Propane-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.0 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

**Diluent Compatibility:** Diluent Buffer A, 50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

## Quality Control Assays

**Ligation:** After 20-fold overdigestion with Sau3AI, approximately 75% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

**16-Hour Incubation:** A 50 µl reaction containing 1 µg of DNA and 20 units of Sau3AI incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

**Exonuclease Activity:** Incubation of 20 units of Sau3AI with 1 µg sonicated $^3H$ DNA ($10^5$ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

**Endonuclease Activity:** Incubation of 10 units of Sau3AI, with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

## Enzyme Properties

**Activity in NEBuffers:**

| | |
|---|---|
| NEBuffer 1 | 100% |
| NEBuffer 2 | 50% |
| NEBuffer 3 | 10% |
| NEBuffer 4 | 100% |

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Survival in a Reaction:** A minimum of 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

**Heat Inactivation:** 65°C for 20 minutes.

**Notes:** Sau3AI and DpnII are isoschizomers of MboI.

Unlike DpnII and MboI, Sau3AI is not blocked by *dam* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

U.S. Patent No. 5,175,101

CERTIFICATE OF ANALYSIS

Sau3AI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R0169S 095120713071

R0169S

NEB1 BSA 37° Yes

200 units Lot: 0951207 Exp: 7/13
RECOMBINANT 4,000 U/ml Store at –20°C

Recognition Site:

5′. . .▼GATC. . . 3′
3′. . . CTAG▲. . . 5′

Source: An *E. coli* strain that carries the cloned Sau3AI gene from *Staphylococcus aureus* 3A (J.S. Sussenbach)

Now Recombinant

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 1, 100X BSA.

Reaction Conditions: 1X NEBuffer 1, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 1:
10 mM Bis Tris Propane-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.0 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A, 50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 20-fold overdigestion with Sau3AI, approximately 75% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 20 units of Sau3AI incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 20 units of Sau3AI with 1 µg sonicated $^3H$ DNA ($10^5$ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 10 units of Sau3AI, with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

| | |
|---|---|
| NEBuffer 1 | 100% |
| NEBuffer 2 | 50% |
| NEBuffer 3 | 10% |
| NEBuffer 4 | 100% |

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Survival in a Reaction: A minimum of 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

Notes: Sau3AI and DpnII are isoschizomers of MboI.

Unlike DpnII and MboI, Sau3AI is not blocked by *dam* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

U.S. Patent No. 5,175,101

CERTIFICATE OF ANALYSIS