Restriction Endonuclease Sac I (Sst I)

From Streptomyces achromogenes

| Cat. No. | 10 669 792 001 | 1000 units (10 U/μl) |
| Cat. No. | 10 669 806 001 | 5000 units (10 U/μl) |
| Cat. No. | 11 047 655 001 | 5000 units, high concentration (40 U/μl) |

**Stability/Storage**

The undiluted enzyme solution is stable when stored at −15 to −25°C until the control date printed on the label. Do not store below −25°C to avoid freezing.

**Sequence specificity**

Sac I recognizes the sequence GAGCT/C and generates fragments with 3’-cohesive termini.

**Compatible ends**

The enzyme has no compatible ends to other known restriction enzymes.

**Isoschizomers**

Sac I is an isoschizomer to Sst I (1).

**Methylation sensitivity**

Sac I is inhibited by the presence of 5-methylcytosine at the central C position, as indicated (*). The presence of 5-methylcytosine at the other C-position or of 6-methyladenine is not inhibiting (*).

**Storage buffer**

20 mM Tris-HCl, 150 mM NaCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.01% polydocanol (v/v), 50% Glycerol (v/v), pH approx. 7.4 (at 4°C).

**Suppl. Incubation buffer, 10x**

330 mM Tris-acetate, 660 mM K-acetate, 100 mM Mg-acetate, 5 mM Dithioerythritol, pH 7.9 (at 37°C), (= SuRE/Cut Buffer A).

**Activity in SuRE/Cut Buffer System**

Bold face printed buffer indicates the recommended buffer for optimal activity:

<table>
<thead>
<tr>
<th>Buffer</th>
<th>A</th>
<th>B</th>
<th>L</th>
<th>M</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>0-10%</td>
<td>100%</td>
<td>50-75%</td>
<td>0-10%</td>
<td></td>
</tr>
</tbody>
</table>

**Incubation temperature**

37°C

**Typical experiment**

<table>
<thead>
<tr>
<th>Component</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1 μg g−1 A−1</td>
</tr>
<tr>
<td>10 × SuRe/Cut Buffer A</td>
<td>5.0 μl</td>
</tr>
<tr>
<td>Nuclease purified water</td>
<td>Up to a total volume of 50 μl</td>
</tr>
<tr>
<td>Restriction enzyme</td>
<td>1 unit</td>
</tr>
<tr>
<td>Incubate at 37°C for 1 h.</td>
<td></td>
</tr>
</tbody>
</table>

**Heat inactivation**

Sac I can be heat-inactivated by 15 min incubation at 65°C (tested up to 100 U/μg DNA).

**Number of cleavage sites on different DNAs (2):**

<table>
<thead>
<tr>
<th>DNA</th>
<th>λ</th>
<th>Ad2</th>
<th>SV40</th>
<th>PhX174</th>
<th>M13mp7</th>
<th>pBR322</th>
<th>pBR328</th>
<th>pUC18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Activity in PCR buffer**

Relative activity in PCR mix (Taq DNA Polymerase buffer) is 100%. The PCR mix contained 1 target DNA, primers, 10 mM Tris-HCl (pH 8.3, 20°C), 50 mM KCl, 1.5 mM MgCl2, 200 μM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

**Ligation and recutting assay**

Sac I fragments obtained by complete digestion of 1 μg λDNA are ligated with 1 unit T4-DNA ligase in a volume of 10 μl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM MgCl2, 5 mM Dithioerythritol, 1 mM ATP, pH 7.5 (20°C) resulting in >95% recovery of 1 μg λDNA fragments. Subsequent re-cutting with Sac I yields >95% of the typical pattern of λDNA × Sac I fragments.

**Troubleshooting**

A critical component is the DNA substrate. Many compounds used in the isolation of DNA such as phenol, chloroform, EtOH, SDS, high levels of NaCl, metals (e.g. Hg2+, Mn2+) inhibit or alter recognition specificity of many restriction enzymes. Such compounds should be removed by EtOH precipitation followed by drying, before the DNA is added to the restriction digest reaction.

Appropriate mixing of the enzyme is recommended.

**Quality control**

Lot-specific certificates of analysis are available at www.roche-applied-science.com/certificates.

Absence of unspecific endonuclease activities

1 μg λDNA is incubated for 16 h in 50 μl incubation buffer with excess of Sac I. The number of enzyme units which do not change the enzyme–specific pattern is stated in the certificate of analysis.

Absence of exonuclease activity

Approx. 5 μg [3H]-labeled calf thymus DNA are incubated with 3 μl Sac I for 4 h at 37°C in a total volume of 100 μl 50 mM Tris-HCl, 10 mM MgCl2, 1 mM Dithioerythritol pH approx. 7.5. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

**References**

3. Rebase The Restriction Enzyme Database: http://rebase.neb.com

www.roche-applied-science.com
Ordering Information


You can view the following manuals on our website:
- Molecular Weight Markers for Nucleic Acids
- Restriction Enzyme Ordering Guide
- Lab FAQs "Find a Quick Solution"
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Changes to previous version
Update of quality control.

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Commonly used bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL21</td>
<td>E. coli B F- dcm ompT hsdS2 (r-g m-1) gal (Studier, F.W. et al., 1986) J. Mol. Biol., 189, 113.</td>
</tr>
<tr>
<td>C600</td>
<td>supE44 hsdR2K2 thi-1 recA1 gyrA96 relA1 thi1 supE44 F[traD36 proAB lacIq lacZ15T3 recA1 endA1 gyrA96 thi1 relA1; (Yanisch-Perron, C. et al., 1985) Gene 33, 103.)</td>
</tr>
<tr>
<td>DH5α</td>
<td>supE44 lacU169 (800lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi1 relA1; (Hanahan, D., 1983) J. Mol. Biol., 166, 557.)</td>
</tr>
<tr>
<td>HB101</td>
<td>supE44 hsdS2O recA13 ara-14 proA2 lacY1 galK2 rpsL20 mtl-1; (Hanahan, D., 1983) J. Mol. Biol., 166, 557.)</td>
</tr>
<tr>
<td>JM108</td>
<td>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi1 supE44 lacIq lacZ15T3 recA1 endA1 gyrA96 thi1 relA1; (Yanisch-Perron, C. et al., 1985) Gene 33, 103.)</td>
</tr>
<tr>
<td>JM109</td>
<td>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi1 supE44 lacIq lacZ15T3 recA1 endA1 gyrA96 thi1 relA1; (Yanisch-Perron, C. et al., 1985) Gene 33, 103.)</td>
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<tr>
<td>JM110</td>
<td>supE44 hsdS2O recA13 ara-14 proA2 lacY1 galK2 rpsL20 mtl-1; (Hanahan, D., 1983) J. Mol. Biol., 166, 557.)</td>
</tr>
<tr>
<td>K802</td>
<td>supE44 hsdS2O recA13 ara-14 proA2 lacY1 galK2 rpsL20 mtl-1; (Hanahan, D., 1983) J. Mol. Biol., 166, 557.)</td>
</tr>
<tr>
<td>SURE2</td>
<td>recB recC sbcC201 uvrC umuC::Tn5(kan) lacI, (hsdRMS) endA1 gyrA96 thi1 relA1 supE44 F[proAB lacIq lacZ15T3 recA1 endA1 gyrA96 thi1 relA1; (Yanisch-Perron, C. et al., 1985) Gene 33, 103.)</td>
</tr>
<tr>
<td>TG1</td>
<td>supE44 hsdS2O recA13 ara-14 proA2 lacIq lacZ15T3 recA1 endA1 gyrA96 thi1 relA1; (Yanisch-Perron, C. et al., 1985) Gene 33, 103.)</td>
</tr>
<tr>
<td>XLI-Blue</td>
<td>supE44 hsdS2O recA13 ara-14 proA2 lacIq lacZ15T3 recA1 endA1 gyrA96 thi1 relA1; (Yanisch-Perron, C. et al., 1985) Gene 33, 103.)</td>
</tr>
<tr>
<td>XLI-Blue</td>
<td>supE44 hsdS2O recA13 ara-14 proA2 lacIq lacZ15T3 recA1 endA1 gyrA96 thi1 relA1; (Yanisch-Perron, C. et al., 1985) Gene 33, 103.)</td>
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