MBRE012

Sac I

Components

<table>
<thead>
<tr>
<th>Reagents provided</th>
<th>MBRE012</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250 Units</td>
</tr>
<tr>
<td>Sac I</td>
<td>6.25 μl</td>
</tr>
<tr>
<td>10X HiBuffer H2</td>
<td>500 μl</td>
</tr>
<tr>
<td>10X HiBuffer DB</td>
<td>500 μl</td>
</tr>
<tr>
<td>Diluent E Buffer</td>
<td>250 μl</td>
</tr>
</tbody>
</table>

NOTE: BSA included in all Reaction Buffer

Source: An E.coli strain that carries the Sac I gene from Streptomyces achromogenes

Recognition Sequence:

5’...GAAGCTC...3’
3’...CTCGAG...5’

Concentration: 40 U/μl

Unit Definition:

1 u is defined as the amount of enzyme that is required to digest 1μg of DNA in 1 hour at 37°C in 50μl of assay buffer.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Optimum reaction temperature (°C)</th>
<th>Thermal Inactivation (°C)</th>
<th>% activity of Buffers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sac I</td>
<td>37</td>
<td>65</td>
<td>H1 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H2 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H3 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H4 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H5 100</td>
</tr>
</tbody>
</table>

Reaction Buffer:

10X HiBuffer H2:
10mM Tris -HCl (pH 7.5 at 30°C), 10mM MgCl₂, 50mM NaCl and 100μg/ml BSA.

NOTE: Incubate at 37°C

Storage Buffer:
10mM Tris-HCl (pH 7.5), 300mM NaCl, 0.1mM EDTA, 7mM 2-mercaptoethanol, 200μg/ml BSA and 50% glycerol. Store at −20°C.

NOTE: 10X HiBuffer DB is provided for double digestion.

Quality Control Assays:

Ligation / Recutting Assay:
After 40-fold over digestion with Sac I, more than 95% of the DNA fragments can be ligated and recut.
Over digestion Assay:
An unaltered banding pattern was observed after 1μg of DNA was digested with 80U of Sac I for 16 hours at 37°C.

Example of Digestion conditions:
- Enzyme concentration : 1 Unit
- Lambda (Hind III digest) 0.3 μg/μl : 3.33 μl (1 μg DNA)
- 10X HiBuffer H2 : 5 μl
- Nuclease free water : upto 50 μl

Note:
- Total reaction volume is dependent on the experiment
- The amount of enzyme to be used is dependent on the DNA template
- For plasmid DNA, 5-10X more enzyme is required
- High enzyme concentration may result in Star activity

Storage conditions: Sac I should be stored at -20°C.