**MBRE013**

**Sca I**

**Components**

<table>
<thead>
<tr>
<th>Reagents provided</th>
<th>MBRE013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250 Units</td>
</tr>
<tr>
<td>Sca I</td>
<td>25 µl</td>
</tr>
<tr>
<td>10X HiBuffer DB</td>
<td>25 µl</td>
</tr>
<tr>
<td>Diluent E Buffer</td>
<td>25 µl</td>
</tr>
</tbody>
</table>

**NOTE:** BSA included in all Reaction Buffer

**Source:** A *E.coli* strain that carries the Sca I gene from *Streptomyces albus*

**Recognition Sequence:**

\[
5'...A\ G\ T\ C\ T...3'
\]
\[
3'...T\ C\ A\ T\ G\ A...5'
\]

**Concentration:** 10 u/µl

**Unit Definition:**

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Optimum reaction temperature (°C)</th>
<th>Thermal Inactivation (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sca I</td>
<td>37</td>
<td>H1 75 H2 75 H3 10 H4 10 H5 75</td>
</tr>
</tbody>
</table>

**Reaction Buffer:**

Buffer 0.5X DB

12.5mM Tris-acetate (pH 7.6 at 30°C), 5mM Mg-acetate, 50mM K-acetate, 3.5mM 2-mercaptoethanol and 25µg/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 10-fold over digestion with Sca I, 70% of the DNA fragments can be ligated and recut.

**Over digestion Assay:**

An unaltered banding pattern was observed after 1mg of DNA was digested with 20U of Sca I for 16 hours at 37°C.
Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda 0.3 mg/ml : 3.33 μl (1 mg DNA)
- 10X HiBuffer DB : 2.5 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: Sca I should be stored at -20°C.