MBRE004

Taq I

Components

<table>
<thead>
<tr>
<th>Reagents provided</th>
<th>MBRE004</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250 Units</td>
</tr>
<tr>
<td>Taq I</td>
<td>12.5 μl</td>
</tr>
<tr>
<td>10X HiBuffer H5</td>
<td>100 μl</td>
</tr>
<tr>
<td>10X HiBuffer DB</td>
<td>100 μl</td>
</tr>
<tr>
<td>Diluent E Buffer</td>
<td>75 μl</td>
</tr>
</tbody>
</table>

NOTE: BSA included in all Reaction Buffer

Source: A *E. coli* strain that carries the Taq I gene from *Thermus aquaticus*

Recognition Sequence:

5’ . . . T C G A . . . 3’

3’ . . . A G C T . . . 5’

Concentration: 20 U/μl

Unit Definition:

1 u is defined as the amount of enzyme that is required to digest 1 μg of DNA in 1 hour at 37°C in 50 μl 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>Taq I</td>
<td>65</td>
<td>None</td>
<td>H1 10  H2 100  H3 75  H4 75  H5 100</td>
</tr>
</tbody>
</table>

Reaction Buffer:

10X HiBuffer H5:

30mM Tris-acetate (pH 7.9 at 30°C), 10mM Mg-acetate, 60mM K-acetate, and 100 μg/ml BSA.

NOTE: Incubate at 65°C

Storage Buffer:

10mM Tris-HCl (pH 7.5), 300mM NaCl, 0.1mM EDTA, 7mM 2-mercaptoethanol, 200 μg/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 20-fold over digestion with Taq I, about 95% of the DNA fragments can be ligated and recut.

Over digestion Assay:

An unaltered banding pattern was observed after 1 μg of DNA was digested with 40U of Taq I for 16 hours at 65°C.
Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda DNA 0.3 μg/μl : 3.33 μl (1 μg DNA)
- 10X HiBuffer H5 : 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: Taq I should be stored at -20°C.