MBRE009  

**EcoR V**

**Components**

<table>
<thead>
<tr>
<th>Reagents provided</th>
<th>MBRE009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250 Units</td>
</tr>
<tr>
<td>EcoR V</td>
<td>12.5 μl</td>
</tr>
<tr>
<td>10X HiBuffer EcoR V</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 EcoR V gene from *Escherichia coli*

**Recognition Sequence:**

5'...G A T A T C...3'

3'...C T A T A G...5'

**Concentration:** 20 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>
<th>% activity of Buffers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H1</td>
</tr>
<tr>
<td>EcoR V</td>
<td>37</td>
<td>None</td>
<td>0</td>
</tr>
</tbody>
</table>

**Reaction Buffer:**

10X HiBuffer EcoR V:

10mM Tris-HCl (pH 8.5 at 30°C), 10mM Mg-Cl₂, 100mM NaCl, and 100 μg/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 20-fold over digestion with EcoR V, 80% 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 40U of EcoR V for 16 hours at 37°C.
Example of Digestion conditions:
- Enzyme concentration : 1 Unit
- Lambda 0.3 μg/ml : 3.33 μl (1 μg DNA)
- 10X HiBuffer EcoR V : 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: EcoR V should be stored at -20°C.