Restriction Enzyme

**Source:** Ruegeria gelatinovora

**Reaction conditions**

1X FastGene® Buffer IV 37℃
1X FastGene® FastCut Buffer, 37℃

**FastGene® Buffer**
FastGene® restriction enzyme can cut substrate DNA in 5-15 min with FastGene® FastCut Buffer.

**1X FastGene® Buffer IV**
20 mM Tris-acetate (pH 7.9 at 25℃)
50 mM potassium acetate
10 mM magnesium acetate
100 μg/ml BSA

**Unit definition**
One unit is defined as the amount of enzyme required for complete digestion of 1 μg bacteriophage λ at 37℃ for 1 hr in 50 μl reaction mixtures.

**Quality control**
- Unit definition assay
- Overdigestion assay
- Endonuclease assay
- Extreme pure assay

**Dilution buffer:** FastGene® Diluent A

**Heat Inactivation**
Age I can be inactivated at 65℃ for 20 min.

**Methylation sensitivity**
dm methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: sensitive

**Prolonged incubation**
A minimum amount of enzyme required to digest 1 μg substrate DNA for 16 hr, 0.25 U.

**Relative activity in FastGene® Buffers**

<table>
<thead>
<tr>
<th>Buffer I</th>
<th>Buffer II</th>
<th>Buffer III</th>
<th>Buffer IV</th>
<th>FastGene® FastCut Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>50%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Note**
Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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**Standard reaction condition**

- **Normal protocol**

<table>
<thead>
<tr>
<th>Component</th>
<th>Final Conc.</th>
<th>Volume</th>
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<tbody>
<tr>
<td>Substrate DNA</td>
<td>1 μg</td>
<td>X μl</td>
</tr>
<tr>
<td>10X FastGene® Buffer IV</td>
<td>1 X</td>
<td>5 μl</td>
</tr>
<tr>
<td>Age I</td>
<td>5 unit</td>
<td>1 μl</td>
</tr>
<tr>
<td>Sterile water</td>
<td>up to 50 μl</td>
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→ Incubate at 37℃ for 1 hr

- **Fast protocol**

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→ Incubate at 37℃ for 15 min

※ We recommend 5-10 units of enzyme per μg DNA and 10-20 units for genomic DNA in a 1 h digest.