**MboII**

**Restriction Endonuclease**

**Recognition Sequence:**

5'-G A A G A (N) 8-3'
3'-C T T C T (N) 7–5'

**Cat. No.**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2284-01</td>
<td>250 units</td>
</tr>
<tr>
<td>E2284-02</td>
<td>1,250 units</td>
</tr>
</tbody>
</table>

**Reaction Temperature:** 37°C

**Inactivation Temperature (20 min):** 65°C

**Prototype:** MboII

**Source:** Moraxella bovis

**Package Contents:**

- MboII
- 10x Reaction Buffer Low
- BSA (100x)

Added as separate component to prevent reaction buffer precipitation.

- Dilution Buffer #1

Added only for enzymes exceeding 10 U/µl in concentration. Use dilution buffer to dilute working stocks of enzyme to a customary concentration of 5 to 10 U/µl. Diluted enzyme stocks will not freeze during storage at -20°C.

**Storage Conditions:** Store at -20°C

**Double Digestion – Buffer Compatibility:**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>% Relative Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>100</td>
</tr>
<tr>
<td>Medium</td>
<td>50</td>
</tr>
<tr>
<td>High</td>
<td>25</td>
</tr>
<tr>
<td>Acet</td>
<td>100</td>
</tr>
</tbody>
</table>

**Recommended Buffer:** Low (or compatible third party buffers)

**DNA Methylation:**

- No inhibition: dcm, EcoKI
- Potential inhibition: dcm, Cpg

**Standard Reaction Protocol:**

Mix the following reaction components:

- 1-2 µg pure DNA or 10 µl PCR product (≈0.1-2 µg DNA)
- 5 µl 10x Buffer Low
- 0.5 µl BSA (100x)
- 1-2 U MboII (use 1 U / µg DNA, < 10 % React. Volume!)

Tips: Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.

- @ 50 µl H₂O, DNA and DNase free

Incubate for 1 h at 37°C

Stop reaction by alternatively:

- (a) Addition of 2.1 µl EDTA pH 8.0 (0.5 M), final 20 mM or
- (b) Heat Inactivation 20 min at 65°C or
- (c) Spin Column DNA Purification (e.g. EURx PCR/DNA CleanUp Kit, Cat.No. E3520) or
- (d) Gel Electrophoresis and Single Band Excision (e.g. EURx AgaroseOut DNA Kit, Cat.No. E3540) or
- (e) Phenol-Chloroform Extraction or Ethanol Precipitation.

**Note:** To avoid star activity it is not recommended:

- to use more than 10 units per reaction;
- to incubate over 1 hr.

**Non-optimal buffer conditions:**

To compensate for the lack of enzyme activity, increase the amount of enzyme and / or reaction time accordingly (but be careful not to cross the mentioned limitations for enzyme amount per µg DNA and for reaction time). The following values may serve as orientation:

- **Enzyme amount:** Instead of 1 U enzyme, use ~4 U of enzyme in buffers providing 25 % rel. activity, ~2 U in 50 %, ~1.5 U in 75 % or ~1 U in 100 %, respectively.
- **Reaction time:** Increase by ~1.3-fold (75 % rel. activity), ~2 fold (50 %) or ~4 fold (25 %), respectively.

**Unit Definition:**

One unit is the amount of enzyme required to completely digest 1 µg of unmethylated Lambda DNA in 1 hr in a total reaction volume of 50 µl. Enzyme activity was determined in the recommended reaction buffer.

**Reaction Buffer:**

1 x Low Buffer: 10 mM Tris-HCl, (pH 7.0 at 37°C), 10 mM MgCl₂, 1 mM dithiothreitol.

To be supplemented with 100 µg/ml bovine serum albumin.

**Reaction Buffer Compatibility:**

Both, enzyme and buffers are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

**Storage Buffer:**

10 mM potassium phosphate (pH 7.0), 50 mM KCl, 0.1 mM EDTA, 7 mM beta-mercaptoethanol, 300 µg/ml bovine serum albumin and 50 % [v/v] glycerol.

**Quality Control:**

All preparations are assayed for contaminating endonuclease, 3’-exonuclease, 5’-exonuclease/5’-phosphatase, as well as nonspecific single- and double-stranded DNase activities.