Thermo SCIENTIFIC

PRODUCT INFORMATION

MboII

#ER0821 300 u

Lot: **Expiry Date:**

5'...**G A A G A (N)$_8$**↓...3'
3'...**C T T C T (N)$_7$**↑...5'

Concentration: 5 u/µl
Source: *E.coli* that carries the cloned *mbolIR* gene from *Moraxella bovis*
Supplied with: 1 ml of 10X Buffer B
1 ml of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/fermentas

RECOMMENDATIONS

1X Buffer B (for 100% MboII digestion)
10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 mg/ml BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of MboII required to digest 1 µg of lambda DNA *dam*⁻ in 1 hour at 37°C in 50 µl of recommended reaction buffer.

Dilution
Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

Double Digests
Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to to www.fermentas.com/doubledigest to choose the best buffer for your experiments.
1X Tango Buffer: 33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

Storage Buffer

MboII is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/ml BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer B	2 µl
DNA (0.5-1 µg/µl)	1 µl
MboII	0.5-2 µl*****

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*****.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µl (~0.1-0.5 µg of DNA)
nuclease-free water	18 µl
10X Buffer B	2 µl
MboII	1-2 µl*****

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*****.

***** *See* Star Activity.

Thermal Inactivation

MboII is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
100	50-100	20-50	0-20	50-100	20-50

Star Activity

An excess of MboII (15 u/µg DNA x 1 hour) may result in star activity.

Methylation Effects on Digestion

Dam: may overlap – blocked.
Dcm: never overlaps – no effect.
CpG: may overlap – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – no effect.

Stability during Prolonged Incubation

A minimum of 1 unit of enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
130	11	11	8	7/8	9	11

For **CERTIFICATE OF ANALYSIS** *see* back page

Note

- MboII is blocked by overlapping *dam* methylation. To avoid *dam* methylation, use a *dam*$^-$, *dcm*$^-$ strain such as GM2163 (#M0099).
- MboII produces DNA fragments that have a single-base 3'-extension which are more difficult to ligate than blunt-ended fragments.
- MboII may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band patterns, use the 6X DNA Loading Dye&SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 10-fold overdigestion with MboII (10 u/µg lambda DNA *dam*$^-$ x 1 hour) (*see* Star Activity).

Ligation/Recutting Assay

After a 5-fold overdigestion (2 u/µg DNA x 2.5 hour) with MboII, more than 80% of the digested DNA fragments can be ligated in a reaction mixture containing 20-40 u of T4 DNA ligase/1 µg of fragments and 10% PEG at a 5'-termini concentration of 1.3 µM. More than 80% of these sites can be recut.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of MboII for 4 hours.

Quality authorized by:  Jurgita Zilinskiene