

**PRODUCT INFORMATION**

# MboII

**#ER0822** 1500 U

**Lot:** \_\_\_\_ **Expiry Date:** \_\_

5'... **G A A G A (N)** <sub>8</sub>↓...3'

3'... **C T T C T (N)** <sub>7</sub>↑...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/onebio](http://www.thermoscientific.com/onebio)

## RECOMMENDATIONS

**1X Buffer B** (for 100% MboII digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 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*<sup>-</sup> 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<sup>™</sup> Buffer. Please refer to [www.thermoscientific.com/doubledigest](http://www.thermoscientific.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

MbolI 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
MbolI	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
MbolI	1-2 µL*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

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\* See Star Activity.

## Thermal Inactivation

MbolI 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 MbolI (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

- MbolI is blocked by overlapping *dam* methylation. To avoid *dam* methylation, use a *dam*<sup>-</sup>, *dcm*<sup>-</sup> strain such as GM2163 (#M0099).
- MbolI produces DNA fragments that have a single-base 3'-extension which are more difficult to ligate than blunt-ended fragments.
- MbolI 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 MbolI (10 U/μg lambda DNA *dam*<sup>-</sup> x 1 hour) (*see* Star Activity).

### Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

### Labeled Oligonucleotide (LO) Assay

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

Quality authorized by:

 Jurgita Zilinskiene

### PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to [www.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio) for Material Safety Data Sheet of the product.

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