Thermo SCIENTIFIC

**PRODUCT INFORMATION**

# MnlI

**#ER1071** 300 U

**Lot: ___** **Expiry Date: _**

5'...**C C T C (N)$_7$**↓...3'
3'...**G G A G (N)$_6$**↑...5'

Concentration: 10 U/µL
Source: *E.coli* that carries the cloned *mnlIR* gene from *Moraxella nonliquefaciens*
Supplied with: 1 mL of 10X Buffer G
1 mL of 10X Buffer Tango

**Store at -20°C**


BSA included

**www.thermoscientific.com/onebio**

## RECOMMENDATIONS

**1X Buffer G** (for 100% MnlI digestion)
10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mg/mL BSA.

**Incubation temperature**
37°C.

**Unit Definition**
One unit is defined as the amount of MnlI required to digest 1 µg of lambda DNA 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 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

MnlI is supplied in: 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.

## Recommended Protocol for Digestion

- Add:

| | |
|---|---|
| nuclease-free water | 16 µL |
| 10X Buffer G | 2 µL |
| DNA (0.5-1 µg/µL) | 1 µL |
| MnlI | 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 G | 2 µL | |
| MnlI | 1-2 µL | |

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

## Thermal Inactivation

MnlI 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 |
|---|---|---|---|---|---|
| 50-100 | **100** | 20-50 | 20-50 | 20-50 | 20-50 |

## Methylation Effects on Digestion

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

## Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of 1 µg of DNA in 16 hours at 37°C.

## Number of Recognition Sites in DNA

| λ | ΦX174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|---|---|---|---|---|---|---|
| 262 | 34 | 26 | 14 | 13 | 12 | 61 |

**Note**

- MnlI produces DNA fragments that have a single-base 3'-extension which are more difficult to ligate than blunt-ended fragments.
- MnlI 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.

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

# CERTIFICATE OF ANALYSIS

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with MnlI (10 U/µg lambda DNA x 16 hours).

## 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 MnlI for 4 hours.

**Quality authorized by:**  Jurgita Zilinskiene