Thermo
SCIENTIFIC

**PRODUCT INFORMATION**

# XhoI

**#ER0691** 2000 U

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

5'...**C↓T C G A G**...3'
3'...**G A G C T↑C**...5'

Concentration: 10 u/µL
Supplied with: 1 mL of 10X Buffer R
1 mL of 10X Buffer Tango

**Store at -20°C**

R | 37° | CG | 20' 80° | HC | | LO

In total 3 vials. BSA included

**www.thermoscientific.com/onebio**

## RECOMMENDATIONS

**1X Buffer R** (for 100% XhoI digestion)
10 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA.

**Incubation temperature**
37°C.

**Unit Definition**
One unit is defined as the amount of XhoI required to digest 1 µg of lambda DNA-HindIII fragments 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**
XhoI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM DTT, 1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

| | |
|---|---|
| nuclease-free water | 16 µL |
| 10X Buffer R | 2 µL |
| DNA (0.5-1 µg/µL) | 1 µL |
| XhoI | 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 R | 2 µL | |
| XhoI | 1-2 µL* | |

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

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* This volume of the enzyme is recommended for preparations of standard concentrations (10 u/µL), whereas HC enzymes (50 u/µL) should be diluted with Dilution Buffer to obtain 10 u/µL concentration.

## Thermal Inactivation

XhoI is inactivated by incubation at 80°C for 20 min.



# ENZYME PROPERTIES

## Enzyme Activity in Thermo Scientific REase Buffers, %

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

## Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – cleavage impaired.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

## Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

## Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

## Compatible Ends

Eco88I, PspXI, SalI, SmoI, SgrDI.

## Number of Recognition Sites in DNA

| λ | ΦX174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 0 | 0 | 0 | 0 |

**Note**

Supercoiled plasmids may require up to 5-fold more XhoI for complete digestion than linear DNA.

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 XhoI (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 occured during incubation with 10 units of XhoI for 4 hours.

## Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

**Quality authorized by:** Jurgita Zilinskiene