Thermo SCIENTIFIC

PRODUCT INFORMATION

XhoI

#ER0695 5000 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: 2 x 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 4 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