

#### 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

 $\mathbf{R}$   $\mathbf{37^0}$   $\mathbf{6}$ 

**CG** 







In total 3 vials.

BSA included

www.thermoscientific.com/onebio

#### **RECOMMENDATIONS**

**1X Buffer R** (for 100% Xhol digestion)

10 mM Tris-HCl (pH 8.5), 10 mM MgCl<sub>2</sub>, 100 mM KCl, 0.1 mg/mL BSA.

## **Incubation temperature**

37°C.

#### **Unit Definition**

One unit is defined as the amount of Xhol required to digest 1  $\mu$ g of lambda DNA-Hindlll fragments in 1 hour at 37°C in 50  $\mu$ L 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 <a href="https://www.thermoscientific.com/doubledigest">www.thermoscientific.com/doubledigest</a> 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**

Xhol 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
Xhol	0.5-2 μL <b>*</b>

- 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  $\mu$ L (~0.1-0.5  $\mu$ g of DNA) nuclease-free water 18  $\mu$ L 10X Buffer R 2  $\mu$ L Xhol 1-2  $\mu$ L\*

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

#### **Thermal Inactivation**

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

Rev.13

#### **Enzyme Activity in Thermo Scientific REase Buffers, %**

В	G	0	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.

EcoKl: never overlaps — no effect. EcoBl: never overlaps — no effect.

## **Stability during Prolonged Incubation**

A minimum of 0.1 units of the enzyme is required for complete digestion of 1  $\mu$ g 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, Sall, Smol, SgrDI.

## **Number of Recognition Sites in DNA**

_	λ	ФХ174	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 Xhol for complete digestion than linear DNA.

<sup>\*</sup> 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.

#### **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.

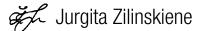
## **Labeled Oligonucleotide (LO) Assay**

No detectable degradation of single-stranded or doublestranded 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:** 





#### 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 <a href="https://www.thermoscientific.com/onebio">www.thermoscientific.com/onebio</a> for Material Safety Data Sheet of the product.

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