



# XhoI

Cat #  RE125S  RE125L

Pack Size:  500U  2000U

Volume:  50  $\mu$ L  100  $\mu$ L

Storage: -20°C

Recognition Sequence: 5' C TCGAG 3'  
3' GAGCT C 5'

## Introduction

XhoI is a commonly used restriction enzyme in molecular biology research. It is classified as a Type II restriction enzyme and is derived from the bacterium *Xanthomonas holcicola*. XhoI recognizes a specific DNA sequence and cleaves it. The recognition sequence for XhoI is 5'-CTCGAG-3'. XhoI is widely employed in molecular cloning and DNA manipulation techniques due to its reliable and efficient digestion properties. It is particularly useful in generating compatible cohesive ends with other restriction enzymes that recognize compatible overhangs, allowing for seamless ligation of DNA fragments.

## Features

- Assayed on  $\lambda$  DNA.
- Ligation/recutting assay: After 20-fold overdigestion with enzyme, >90% of the DNA fragments can be ligated and recut.
- Overdigestion assay: No nonspecific activity detected after incubation of 1  $\mu$ g of  $\lambda$  DNA with 20 units of XhoI for 16 hours.

## Kit content:

S.No	Component	RE125S	RE125L
1	XhoI	500U/50 $\mu$ L(10U/ $\mu$ L)	2000U/100 $\mu$ L(20U/ $\mu$ L)
2	10X Universal Buffer	500 $\mu$ L	2 mL

## Protocol:

- The enzyme should not exceed 10 % of total reaction volume.
- Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex
- Incubate 60 min. at 37 °C.

S.No	Component	50 $\mu$ l Reaction
1	DNA	1 $\mu$ g
2	10X Universal Buffer	5 $\mu$ L
3	XhoI	2-5 units
4	Nuclease-free Water	to 50 $\mu$ L

## Enzyme Inactivation:

Stop reaction by alternatively:

- Addition of 2.1  $\mu$ l EDTA pH 8.0 [0.5 M], final 20 mM
- Heat Inactivation (20 min. at 65 °C)
- Spin Column DNA Purification (e.g. Cat.-No. [PUR13-50](#))
- Gel Electrophoresis and Single Band Excision (e.g. Cat.-No. [PUR12-50](#))
- Phenol-Chloroform Extraction or Ethanol Precipitation.

**Supplied in :** 10mM Tris-Hcl (pH 7.6), 50mM NaCl, 0.1mM EDTA, 1mM DTT, 100 $\mu$ g/ml BSA, 50% Glycerol.

**Unit definition :** One unit is defined as the amount of restriction enzyme required to completely digest 1  $\mu$ g of lambda DNA in 1 hour at 37°C in a total reaction volume of 50  $\mu$ L.

### Quality Control Assays:

- Ligation of DNA fragments: DNA fragments are produced by an excessive over digestion of substrate DNA with each restriction endonuclease. These fragments are then ligated with T4 DNA Ligase at a 5' termini concentration of 0.1-1.0  $\mu\text{M}$ . The ligated fragments are then recut with the same restriction endonuclease. Ligation can only occur if the 3' and 5' termini are left intact, and only those molecules with a perfectly restored recognition site can be recleaved. A normal banding pattern after cleavage indicates that both the 3' and 5' termini are intact, and the enzyme preparation is free of detectable exonucleases and phosphatases.
- DNA digestion with restriction enzymes may be affected by some types of methylation.
- In general, it is recommended to use 5–10 units of enzyme per  $\mu\text{g}$  of plasmid DNA, and 10–20 units for genomic DNA in a 1-hour digest. Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity.
- All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5' exonuclease/ 5' phosphatase, as well as nonspecific single- and doublestranded DNase activities.