Gene to Protein Pvt. Ltd.
www.genetoprotein.com
info@genetoprotein.com
800 GENOME, 800 GENETIC

XbaI

Cat # RE141
Pack Size: 500U
Volume: 50 µL
Storage: -20°C

Recognition Sequence:

5' T↓CTAGA 3'
3' AGATC↑T 5'

Kit content:

S.No	Component	RE141
1	XbaI	500U/50µL(10U/µL)
2	10X Universal Buffer	500 µL

Introduction

XbaI Restriction Enzyme is a high-quality enzyme widely used in molecular biology applications for DNA manipulation and cloning purposes. It belongs to the Type II restriction enzyme family and is derived from the bacterium *Xanthomonas badrii*. XbaI specifically recognizes the DNA sequence and cleaves it, generating cohesive or sticky ends.

Features

- Assayed on λ 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 µg of λ DNA with 20 units of XbaI for 16 hours.

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 µl Reaction
1	DNA	1 µg
2	10X Universal Buffer	5 µL
3	XbaI	2-5 units
4	Nuclease-free Water	to 50µL

Enzyme Inactivation:

Stop reaction by alternatively:

- Addition of 2.1 µl 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, 100µg/ml BSA, 50% Glycerol.

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

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 µM. 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 µg 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.