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

XmaI

Cat # RE127
Pack Size: 500U
Volume: 50 µL
Storage: -20°C

Recognition Sequence: 5' C|CCGGG3'
3' GGGCC|C5'

Kit content:

S.No	Component	RE127
1	XmaI	250U/50µL(5U/µL)
2	10X Universal Buffer	250 µL

Introduction

XmaI is a widely used Type II restriction enzyme that recognizes and cleaves DNA at a specific recognition sequence. The recognition sequence for XmaI is 5'-C/CCGGG 3'. One of the key features of XmaI is its ability to generate cohesive or sticky ends upon DNA digestion. The cleavage of DNA by XmaI leaves 5' overhangs with a sequence of 5'-C/CCGGG -3' on one strand and its complementary sequence on the other strand. These sticky ends can then be easily ligated with other DNA fragments that have complementary overhangs generated by the same enzyme or compatible enzymes. The cohesive end property of XmaI allows for efficient and precise DNA cloning.

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 XmaI 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	XmaI	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.