DATA SHEET

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Xba I

Speedy Restriction Enzyme

Cat. N°.	Amount
☐ ENZ-109XS	1.500 units
☐ ENZ-109S	3.000 units
☐ ENZ- 109M	7.500 units
☐ ENZ- 109L	15.000 units

Unit Definition: One unit is the amount of enzyme required to completely digest 1 µg of Lambda DNA (dam-/Hind III digest; 1 sites) in 1 hour in a total reaction volume of 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Source:
Klebsiella pneumoniae Ok8

For *in vitro* use only!

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C

Additional Storage Conditions:
Avoid freeze/thaw cycles.

Shelf Life:
12 months

Form:
Liquid (Supplied in 10 mM Tris-HCl pH 7.4, 100 mM KCl, 0.1mM
EDTA, 1 mM DTT, 500 µg/ml BSA and 50 % [v/v] glycerol).

Concentration:
10 units/µL

Supplied with:
10X Universal Buffer (UB)

Recommended 50 µL assay:

Components	50 µL rxn
10X Universal Buffer (UB)	To 50 µl
Pure DNA[1] or PCR Product[2]	1 µg
Enzyme	10 units
PCR Grade water	fill up to 50 µL

[1] Supercoiled or high molecular weight DNA (e.g. plant genomic DNA) may require longer incubation time or higher amount of enzyme.
[2] Some enzymes may require additional DNA bases flanking the restriction site for complete digestion.

Double Digestion - Buffer Compatibility

B1 - 50-75% Relative Activity
B2 - 100% Relative Activity
B3 - 75% Relative Activity
B4 - 75% Relative Activity
B5 - 75% Relative Activity
1x UB - 100 % Relative Activity (recommended)

Please note that the optimum digestion condition for this enzyme is 1x UB. Within the Universal Buffer (UB) system, the most majority of our enzymes display 100% Relative Activity in 1x UB and only few either in 0.5x or 2x UB. If optimum condition for second enzyme is different than the recommended for the first enzyme, we suggest carrying out first the restriction at the higher recommended concentration of UB and dilute the reaction volume to the adequate UB concentration for further proceeding with the second restriction.

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 5 to 10 min at 37 °C.
- Stop reaction by alternatively:
  - Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM
  - Heat Inactivation (20 min. at 80 °C)
  - Spin Column DNA Purification (e.g. PCR Purification Kit, Cat. #DPK-106)
  - Gel Electrophoresis and Single Band Excision (e.g. Agarose Gel Extraction Kit, Cat.#DPK-105)
  - Phenol-Chloroform Extraction or Ethanol Precipitation.

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Reaction Enzymes Buffer Guide:

Buffer 1	10X B1	100 mM Tris-HCl (pH 7.9, 25 °C) 100 mM $MgCl_2$ 1000 µg/mL BSA
Buffer 2	10X B2	100 mM Tris-HCl (pH 7.9, 25 °C) 100 mM $MgCl_2$ 500 mM NaCl 1000 µg/mL BSA
Buffer 3	10X B3	500 mM Tris-HCl (pH 7.9, 25 °C) 100 mM $MgCl_2$ 1000 mM NaCl 1000 µg/mL BSA
Buffer 4	10X B4	100 mM Tris-HCl (pH 7.9, 25 °C) 100 mM $MgCl_2$ 1500 mM NaCl 1000 µg/mL BSA
Buffer 5	10X B5	200 mM Tris-acetate (pH 7.9, 25 °C) 100 mM Mg-acetate 500 mM K-acetate 1000 µg/mL BSA

Reaction Buffer Compatibility:

Our restriction enzymes are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

Ligation and recutting:

After 50-fold overdigestion with Xba I, >98% of the DNA fragments can be ligated and recut with this enzyme.

Star activity:

Conditions of low ionic strength, high enzyme concentration, glycerol concentration >5 % or pH >8.0 may result in star activity.

DNA Methylation:

No Inhibition: dcm, CpG
Inhibition (Blocked by overlapping): dam

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/ 5'-phosphatase, as well as nonspecific single- and double stranded DNase activities.