Genenase I™

1-800-632-7799
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www.neb.com

P8075S 0191206140611

P8075S

50 µg **1 µg/µl** **Lot: 0191206**
RECOMBINANT **Store at –20°C** **Exp: 6/14**

Recognition Site:

His-Tyr▼or Tyr▼-His

Description: Genenase I is a variant of subtilisin BPN´ that has been engineered to have increased specificity by substituting amino acids in its active site (1,2). When designing fusion proteins for cleavage with Genenase I, we recommend the site Pro-Gly-Ala-Ala-His-Tyr. Genenase I will cleave at other histidine residues depending on the surrounding amino acids and the 3-dimensional conformation of the protein. Genenase I cleaves His-Tyr-Glu and His-Tyr-Asp slowly, but will not cleave His-Tyr-Pro or His-Tyr-Ile (2).

Source: Isolated from a *Bacillus subtilis* carrying the protease gene derived from *Bacillus amyloliquefaciens.*

Supplied in: 20 mM Tris-HCl, 200 mM NaCl, 2 mM $CaCl_2$, 1 mM DTT, 50% glycerol (pH 7.2 @ 4°C).

Molecular Weight: The molecular weight of Genenase I is 27.4 kDa. Its apparent molecular weight in SDS-PAGE gels is 28 kDa.

Unit Definition: 0.5 µg of Genenase I will cleave 50 µg of test substrate to 95% completion in 8 hours or less at 23°C.

Unit Assay Conditions: 20 mM Tris-HCl (pH 8.0 @ 25°C), 200 mM NaCl, 50 µg of MBP fusion test substrate and enzyme. Incubate at 23°C.

Notes on Reaction Conditions: The rate of Genenase I cleavage has been shown to depend on the amino acid following the cleavage site (position P1')(3). Rates of cleavage when P1' is Asp or Glu are slowest, 40-fold slower than when P1' is Arg or Cys. The rate when P1'= Glu or Asp is enhanced 10- and 14-fold, respectively, by including 2 M KCl in the reaction.

References:

1. Carter, P. and Wells, J.A. (1987) *Science* 237, 394–399.
2. Carter, P et al. (1991) *Biochemistry* 30, 6142–6148.
3. Carter, P. et al. (1989) *Proteins: Structure, Function, and Genetics*I 6, 240-248.

CERTIFICATE OF ANALYSIS

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U.S. Patent No. 5,371,008 and 5,371,190

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