Strem Chemicals, Inc. www.strem.com

Catalog # 06-3160 Neutrase® 0.8 L

Note: Sold in collaboration with Novozymes A/S for research purposes only.

Neutrase® 0.8 L is a neutral, zinc metallo endo-protease from Bacillus amyloliquefaciens that randomly hydrolyses internal peptide bonds. The product also facilitates enzymatic synthesis of oligopeptides by the reverse proteolysis reaction with zinc metal as a co-catalyst. Neutrase® 0.8 L belongs to the same protease family as thermolysin, a zinc dependent metallo endo-protease.

Thermolysin in an immobilized form has been used successfully in industrial processes for synthesis of an Aspartame intermediate. The reaction takes place in organic solvent and involves kinetic resolution of an amine methylester with high enantioselectivity and high regioselectivity in the amide bond formation of the α-carbonyl in aspartic acid preferred over the β-carbonyl.

Declared activity 0.8 AU/g. Kinetic resolution of amino esters is a neutral, zinc metallo endoprotease, that randomly hydrolyzes internal peptide bonds and also facilitates enzymatic synthesis of oligopeptides by the reverse proteolysis reaction with zinc metal as co-catalyst.

Other Endoproteases products offered by Strem:

06-3112 Alcalase® 2.5 L
06-3110 Alcalase® 2.4 L FG
06-3137 Savinase® 12 T
06-3115 Esperase® 8.0 L
06-3150 Savinase® 16L
96-0224 Novozymes Endoprotease Screening Kit (contains 6 endoprotease enzymes)

Lipase products offered by Strem:

06-3123 Novozym® 435
06-3155 Lipozyme® TL IM
06-3120 Novozym® 40086
06-3105 Lipozyme® CALB L
06-3118 Palatase® 20000 L
06-3140 Lipozyme® TL 100 L
06-3125 Resinase® HT
06-3135 Novozym® 51032
06-3100 Novocor® AD L
96-0220 Novozymes Lipase Screening Kit (contains 9 lipase enzymes)

Novozymes Protease Products

Storage

Kits should be optimally stored at 0-10°C/32-50°F. If stored above 25°C/77°F the samples should be used within 3 months.

Introduction

Proteases (EC 3.4.21.62) belong to the class of enzymes known as hydrolases, which catalyze hydrolysis of various bonds in presence of water. Proteases are also referred to as Peptidases or Proteinases. Proteases catalyze proteolysis of peptide bonds in polypeptides, proteins and selective hydrolysis of carboxylic esters and amino esters. There are different classes of Proteases, i.e. serine, threonine, cysteine, aspartate, glutamic acid and metallo – proteases.

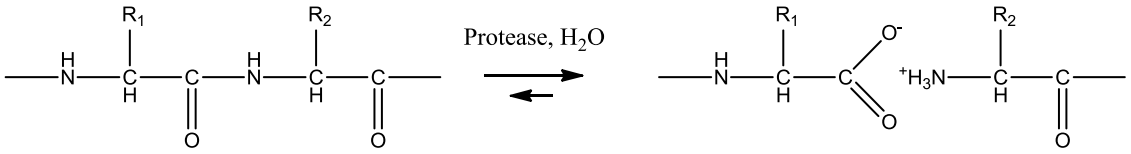


Figure 1. Proteolysis of a peptide bond.

Description and optimum usage conditions

Strem Catalog Number	Product	EC No.	Specifity	Format	Optimal Conditions	Unit Activity	Application
06-3110	Alcalase® 2.4 L FG	3.4.21.62	Serine endopeptidase (mainly subtilisin A)	Liquid	30-65°C, pH 7-9	2.4 AU-A/g	Stereoselective hydrolysis of amino esters and selective esters; suitable for hydrolysis of proteins; used in transesterification and transpeptidation.
06-3112	Alcalase® 2.5 L	3.4.21.62	Serine endopeptidase (mainly subtilisin A)	Liquid	30-65°C, pH 7-10	2.5 AU-A/g	Stereoselective hydrolysis of amino esters and selective esters; suitable for hydrolysis of proteins; used in transesterification and transpeptidation.
06-3137	Savinase® 12 T	3.4.21.62	Serine endopeptidase (mainly subtilisin A)	Granulate	30-70°C, pH 8-10	12 KNPU-S/g	Stereoselective hydrolysis of amino esters and selective esters; suitable for hydrolysis of proteins, hydrolysis of strained amides
06-3150	Savinase® 16 L	3.4.21.62	Serine endopeptidase (mainly subtilisin A)	Granulate	30-70°C, pH 8-10	16 KNPU-S/g	Stereoselective hydrolysis of amino esters and selective esters; suitable for hydrolysis of proteins, hydrolysis of strained amides
06-3115	Esperase® 8.0 L	3.4.21.62	Serine endopeptidase (mainly subtilisin A)	Liquid	pH 8-12.5	8 KNPU-E/g	Hydrolysis of internal peptide bonds; characterized by excellent perfomance at elevated temperature and pH.
06-3160	Neutrase 0.8 L	3.4.22	Metalloprotease	Liquid	40-50°C, pH 7	0.8 AU/g	Kinetic resolution of amino esters

* K = Kilo, AU = Anson Unit, NPU = Novo Protease Unit, 1 AU = 1NPU, ASNU = Asparaginace Unit, USP = Trypsin activity unit using USP Crystallized Trypsin Reference Standard

The activity is determined relative to a protease A standard. The result is given in the same units as the standard.

1 ASNU is the amount of enzyme that produces 1 µmol Ammonia per minute under the standard reaction conditions.

Screening Procedure

Listed below is recommended equipment for conducting the screens, however, pH-stat system gives more consistent results.

Simple equipment	Advanced equipment
Reaction vessel (e.g., 25 mL round bottom or Erlenmeyer flask or test tubes)	Thermostat reaction vessel (e.g., 25 mL)
pH-meter or pH-paper (range 5 - 9)	Autotitrator/pH-stat system (pH-meter, automatic burette/addition funnel)
Burette or calibrated addition funnel	Recording device (e.g., x/y-plotter)
Propeller mixer or magnetic needle	Propeller mixer

Buffer Preparation

0.1M Potassium Phosphate Buffer at 25°C		
pH	Volume of 1M K_2HPO_4 (ml)	Volume of 1M KH_2PO_4 (ml)
5.8	8.5	91.5
6.0	13.2	86.8
6.2	19.2	80.8
6.4	27.8	72.2
6.6	38.1	61.9
6.8	49.7	50.3
7.0	61.5	38.5
7.2	71.7	28.3
7.4	80.2	19.8
7.6	86.6	13.4
7.8	90.8	9.2
8.0	94.0	6.0
Dilute combined 1M stock solutions to 1 L with distilled H_2O.		

0.1M Sodium Phosphate Buffer at 25°C		
pH	Volume of 1M Na_2HPO_4(ml)	Volume of 1M NaH_2PO_4 (ml)
5.8	7.9	92.1
6.0	12.0	88.0
6.2	17.8	82.2
6.4	25.5	74.5
6.6	35.2	64.8
6.8	46.3	53.7
7.0	57.7	42.3
7.2	68.4	31.6
7.4	77.4	22.6
7.6	84.5	15.5
7.8	89.6	10.4
8.0	93.2	6.8
Dilute combined 1M stock solutions to 1 L with distilled H2O.		

Analytical method principles

In-process reaction monitoring:

Depending on substrate and product, different methods can be used for in process reaction monitoring.

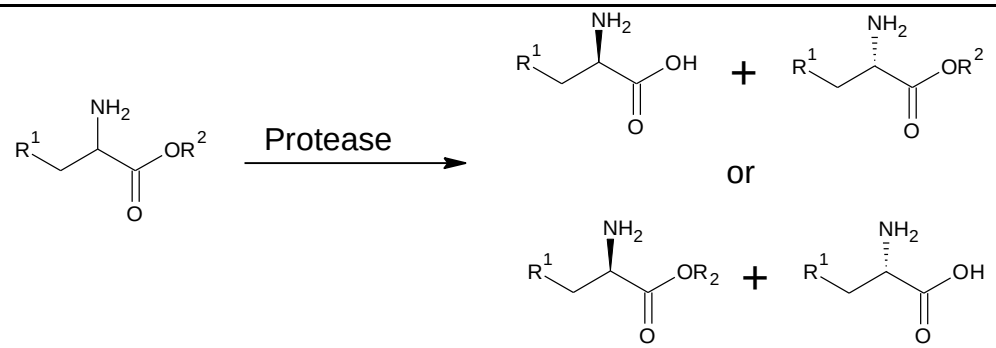
- Thin Layer Chromatography (TLC) is a simple method for monitoring reaction progress and completion.
- To quantitatively estimate product formation and consumption of reactant, HPLC or GC can be used for monitoring.
- Chiral HPLC is recommended to estimate chiral purity or consumption of isomers of racemic mixture.
- Final chiral purity can be obtained by analyzing product isolated by using an appropriate chiral column.

Key parameters for Enantiomeric excess (ee) and Enantioselectivity (E) can be calculated from the areas in chiral HPLC: % ee = ((R-S)/(R+S)) × 100 where R and S stand for the individual optical isomer in the mixture (and R +S = 1), where R = area for R isomer and S = area for S isomer.

$$E = \frac{\ln\left[\dfrac{1 - e.e._s}{1 + e.e._s / e.e._p}\right]}{\ln\left[\dfrac{1 + e.e._s}{1 + e.e._s / e.e._p}\right]}$$

Kinetic Resolution

Example 1. Kinetic Resolution by hydrolysis of Racemic Amino Ester/ Simple Racemic Ester [1, 2]

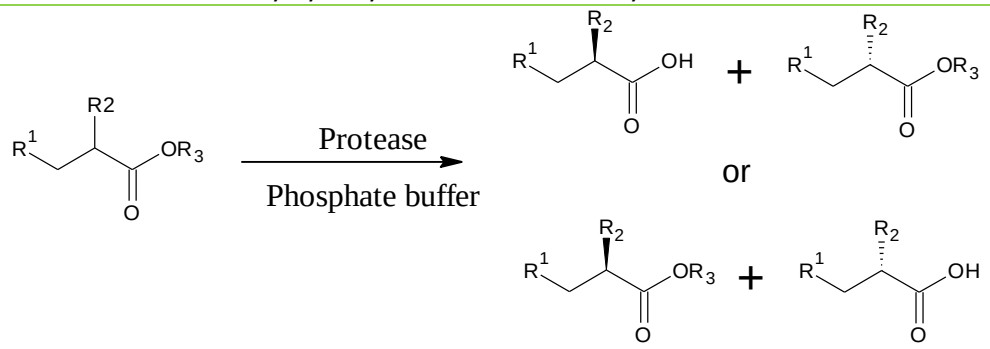


- Racemic amino ester / simple racemic ester (1-2 mmol) is solubilized in potassium phosphate buffer (0.1 M, 10 mL, pH 7.5) and reaction mixture is homogenized by stirring.
  - For liquid substrates, emulsion or suspension will be formed.
  - For solid substrates, solution is prepared by adding 10%v/v organic solvent*
- Protease Enzyme (50% wt/wt for solid enzyme or 10-20% v/v wrt to buffer for liquid enzyme) is added to reaction mixture.
- Reaction mixture is maintained at pH 7.5 by adjusting with 1N NaOH.
- Typical reaction temperature is 25-30°C and reaction time is 24-48 hours, depending on substrate.
- The reaction product is recovered by extraction or filtration.

* isopropanol (IPA), tert-butanol, tetrahydrofuran (THF) or acetonitrile.

Kinetic resolution of racemic amino ester can be converted into dynamic kinetic resolution leading to formation of catalytic quantities of aldehydes leading to formation of one of chiral amino acids in good yields.[2]

Example 2. Kinetic resolution by hydrolysis of racemic carboxylic ester



- Racemic ester (1-2 mmol) and potassium phosphate buffer (0.1 M, pH 7.0, 5 mL) is homogenized by stirring.
- Protease Enzyme (50% wt/wt with regards to substrate for solid enzyme or 10-20% v/v with regards to solvent mixture for liquid enzyme) is added under stirring.
- Reaction mixture is maintained at pH 7.0-9.0 by adjusting with 1N NaOH.
- Typical reaction temperature is 20-35°C and reaction time is 24-48 hours, depending on substrate.
- Reaction product is recovered by extraction or filtration.

References

1. S.-T. Chen, W.-H. Huang, K.-T. Wang, *J. Org. Chem.* **1994**, *59*,7580–7581.

2. D. A. Schichl, S. Enthaler, W. Holla, T. Riermeier, U. Kragl and M. Beller *Eur. J. Org. Chem.* **2008**, 3506–3512

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