Product Information

Protease from *Streptomyces griseus*

Type XIV, ≥3.5 units/mg solid, powder

P5147

Product Description

CAS Registry Number: 9036-06-0

Synonyms: Actinase E, Pronase E

'Pronase E' is the name given to a group of proteolytic enzymes produced by *Streptomyces griseus* K-1. Pronase E contains at least 10 proteases, including:[1]

- five serine-type proteases
- two zinc endopeptidases
- two zinc leucine aminopeptidases
- one zinc carboxypeptidase

Digestion with Pronase has been useful when extensive or complete degradation of protein is required. Pronase digestion is particularly useful since Trp, Ser, Thr, Asn and Glu are easily destroyed by standard acid hydrolysis procedures. This protease mixture is so non-specific that it can digest casein to the extent of >70%, down to mono-amino acids.[1] Pronase has been shown to be much more effective in digestion of casein than trypsin, chymotrypsin and several other proteases.[2]

Structure

This product features at least three caseinolytic activities and one aminopeptidase activity. The caseinolytic enzymes were named as *Streptomyces griseus* Protease A, *Streptomyces griseus* Protease B and *Streptomyces griseus* Trypsin.[3] Several amino acid sequences and molecular weights have been reported:

- 18,093 for Protease A[4]
- 18,629 for Protease B[5]
- 22,918 for *S. griseus* Trypsin.[6] Properties of this trypsin have also been reported.[7]

Values of 16,000 and 18,000 for two different proteolytic activities have been reported.[8] Molecular weights, usually determined by gel filtration, range from 16,000 to 27,000. Additional references on isolation, properties and structure are noted.[14-18]

Kinetic Parameters

Pronase E is highly stable in the pH range 5.0 to 9.0, with optimum activity at pH 7-8.[1] The product has variable stability aspects at < pH 4 and at > pH 10:

- The neutral components in the enzyme mixture are stable at pH 5-9 with calcium present.
- The alkaline components are stable over pH 3-9, with optimal activity at pH 9-10.
- The aminopeptidase and carboxypeptidase components are stable at pH 5-8, in the presence of calcium ion.[1]

The product can be completely inactivated by heating above 80 °C for 15-20 minutes.[1] Some components of the mixture are inactivated more quickly than others. Adding excess EDTA results in irreversible loss of about 70%. The mixture retains activity in 1% SDS (w/v) and 1% Triton (w/v).

Inhibitors

No single substance will inhibit all the different proteases in Pronase. Diisopropylfluorophosphate, PMSF and EDTA have been used with some success.

Method of Preparation

This mixture of proteases is produced from a culture broth of *Streptomyces griseus* and is isolated chromatographically.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The recommended storage temperature for this product is –20 °C. The lyophilized powder is extremely stable if stored frozen and dry.

Solubility/Solution Stability

The product dissolves in 0.01 M sodium acetate with 0.005 M calcium acetate at pH 7.5 at 37 °C. Calcium ion is recommended for protection from autolysis.

The activity of a dilute enzyme solution containing 0.01 M – 0.1 M calcium ion was stable over 24 hours at neutral pH at 2-8 °C. Pronase E is stable at 4 °C for at least six months. Stock solutions of 5 to 20 mg/mL in water are usually stored at –20 °C.[1]

Usage

DNA isolation[1]

Prior to storage at -20 °C, Pronase stock solution is first heated to 56 °C for ~15 minutes, then incubated at 37 °C for 1 hour. This encourages self-digestion, to eliminate DNase and RNase contamination.

The enzyme is added to a DNA sample, in the presence of 0.5-1% SDS to disrupt DNA-protein interactions, typically at 250-500 µg protein/mL, then incubated at 37 °C for 1-4 hours.

Protein hydrolysis

Dissolve about 0.2 micromole of protein in 0.2 mL of 50 mM ammonium bicarbonate buffer at pH 8 (or phosphate buffer pH 7). Add Pronase to 1% (w/w) and incubate at 37 °C for 24 hours. It may be necessary to add aminopeptidase M at 4% (w/w) and incubate at 37 °C for another 18 hours.

Additional applications include:

- hydrolysis of amino acid amides[15]
- pretreatment of liver tissue sections to enhance the intensity of immunostaining[16]
- regeneration of certain types of affinity columns[17]
- removal of protein in DNA/RNA isolations[18]

Several theses[19-21] and dissertations[22-36] cite use of P5147 in their research protocols.

Unit Definition

One unit will hydrolyze casein to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per minute at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

Activity: ≥ 3.5 units protease per mg solid

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