Product Data Sheet

Product Name: Pronase E (Activity ≥ 7000 U/g)
Cat. No.: GC30124

Chemical Properties

Cas. No.	9036-06-0		
SMILES	[Pronase E]		
Formula	$C_{24}H_{28}FN_3O_2.HCl$	M.Wt	445.96
Solubility	DMSO : 50 mg/mL ;Water : 20 mg/mL	Storage	Store at -20°C,protect from light
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Pronase E

Protocol

Pronase E Usage Instructions [1]:

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Pronase E is highly stable within the pH range of 5.0-9.0, with maximum enzyme activity at pH 8.8. Compatible with many isolation buffer systems. Pronase E has broad substrate specificity; can degrade proteins to individual amino acids. Complete inactivation can be achieved by heating to 80 °C or higher for 15-20 minutes.

Unit Definition: Under conditions of 37 °C and pH 7.5, the enzyme quantity required to produce a color change equivalent to 1 μM of tyrosine (181 g of casein) per minute, as measured by the Folin-Phenol reagent method, is defined as one activity unit.

This product is widely used. Specific usage methods and working concentrations should be adjusted based on experimental experience or reference literature.

Preparation of Stock Solution: Dissolve in deionized water to prepare a stock solution with a concentration of 5-20 mg/ml. If used in DNA or RNA isolation steps, it is recommended to heat the solution at 56°C for 15 minutes and then incubate at 37°C for 1 hour. Divide the stock solution into single-use aliquots and store at -20°C; typically stable for one year.

DNA Isolation: Directly add to the DNA preparation system (containing 0.5-1% SDS to disrupt DNA-protein interactions). The typical working concentration range is 250-500 μg protein/ml, with incubation at 37°C for 1-4 hours.

Protein Digestion: Dissolve approximately 0.2 μM of protein in 0.2 ml of 50 mM ammonium bicarbonate buffer, pH 8.0 (or phosphate buffer, pH 7.0). Then, add **Pronase E** at 1% (w/w) and incubate at 37°C for 24 hours.

Protocol for Isolation of primary hematopoietic stem cells（HSCs) in mice by Pronase E [2]:

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1. Male or female mice were anesthetized, and abdominal cavities were opened.
2. The liver was perfused with Hanks' Buffered Salt Solution (HBSS) through portal veins, followed with **Pronase E** and collagenase perfusion.
3. The liver was then gently removed with sterile forceps and transferred into sterile dishes.
4. The hepatic capsule was teared apart, and the liver was gently shaken to release cells.
5. The cells were suspended in solution containing **Pronase E**, collagenase and DNase, then digested at 37°C for 20 min.
6. Hepatocytes were isolated using two rounds of centrifugation at 50g for 3 min.
7. The supernatant was further centrifuged to obtain HSCs at 450g for 8 min, then precipitates were suspended with 18% Nycodenz solution to prepare density gradient centrifugation with 12% Nycodenz, 8.2% Nycodenz and Gey's Balanced Salt Solution (GBSS).
8. Eventually, cells were purified from the layer of 8.2% Nycodenz to obtain HSCs.

This protocol only provides a guideline, and should be modified according to your specific needs.

References:

[1]. Pronase E significantly increases the amount of most of the amino acids analysed (PE vs C) , especially Ile, His and Thr

[2]. Chen QT, Zhang ZY, et,al. HK1 from hepatic stellate cell-derived extracellular vesicles promotes progression of hepatocellular carcinoma. Nat Metab. 2022 Oct;4(10):1306-1321. doi: 10.1038/s42255-022-00642-5. Epub 2022 Oct 3. PMID: 36192599; PMCID: PMC9584821.

Background

Pronase E is a proteolytic enzyme mixture obtained from Streptomyces griseus[1]. Pronase E can break down proteins into individual amino acids, making it suitable for extensive protein degradation. Pronase E is used in various applications, including the study of protease inhibitors, thermal inactivation kinetics, nucleic acid isolation[2], tissue digestion in combination with collagenase and trypsin[3-5], and the purification of glycoproteins for glycopeptide production.

References:

[1]. Pronase E significantly increases the amount of most of the amino acids analyzed (PE vs C),

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especially Ile, His, and Thr
[2]. Zhao W, Xu W, et, al. Key Amino Acid Residues of Mitochondrial Transcription Factor A Synergize with Abasic (AP) Site Dynamics To Facilitate AP-Lyase Reactions. ACS Chem Biol. 2023 May 19;18(5):1168-1179. doi: 10.1021/acschembio.3c00047. Epub 2023 Mar 17. PMID: 36930463; PMCID: PMC10198963.
[3]. Chen QT, Zhang ZY, et, al. HK1 from hepatic stellate cell-derived extracellular vesicles promotes the progression of hepatocellular carcinoma. Nat Metab. 2022 Oct;4(10):1306-1321. doi: 10.1038/s42255-022-00642-5. Epub 2022 Oct 3. PMID: 36192599; PMCID: PMC9584821.
[4]. Fang Z, Xu Y, et, al. Narirutin activates TFEB (transcription factor EB) to protect against Acetaminophen-induced liver injury by targeting PPP3/calcineurin. Autophagy. 2023 Aug;19(8):2240-2256. doi: 10.1080/15548627.2023.2179781. Epub 2023 Feb 24. PMID: 36779633; PMCID: PMC10351474.
[5]. Chen S, Li S, et , al. Caspase-mediated LPS sensing and pyroptosis signaling in Hydra. Sci Adv. 2023 Jul 21;9(29):eadh4054. doi: 10.1126/sciadv.adh4054. Epub 2023 Jul 21. PMID: 37478191; PMCID: PMC10361584.

Caution: Product has not been fully validated for medical applications. For research use only.
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