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PRODUCT INFORMATION
CI*zyme*™ Collagenase HA
Catalog Number: 001-1000

Product Description

CI*zyme*™ Collagenase HA is an aseptically filled, lyophilized mixture of 60% purified class I (C1) and 40% purified class II (C2) collagenase from *Clostridium histolyticum*. This product is purified from culture supernatants of *C. histolyticum* that contain porcine gelatin and pancreatic enzymes derived from US and Canadian sources. No bovine derived animal products are used in any step of the fermentation or purification process. CI*zyme*™ HA is formulated to contain a sufficient amount of collagen degradation activity (CDA) units for the islet isolation applications described below and specific details for each lot appear on a Certificate of Analysis. The purification process ensures that a negligible amount of trypsin like activity (i.e., contaminating clostripain) is present in the product.

Storage and Stability

This product is stable for at least two years from date of manufacture if stored unopened between -15 to -25°C. Internal studies have shown the reconstituted enzyme is stable as a frozen solution between -15 to -25°C for at least 1 year as long as no other protease enzymes had been added to the solution. Additional studies have shown the reconstituted collagenase was successfully frozen and thawed three times as a concentrated or dilute solution without apparent loss of potency as assessed by the CDA assay. The product is shipped on dry ice to provide the most stable conditions during shipment.

Enzyme Preparation

The product is supplied as a lyophilized cake in vacuum sealed amber bottles. Reconstitute the lyophilized enzyme with 20 mL of cold water on ice for a minimum of 30 minutes to ensure complete dissolution of the enzyme. Occasionally invert the vial to aid in the dissolution process. The enzyme solution should not be vortexed or swirled excessively as enzyme denaturation may occur. Failure to allow the enzyme to completely rehydrate will affect the enzyme potency and could negatively impact the success of the tissue dissociation procedure. The enzyme is lyophilized in a buffer containing calcium so the initial reconstitution has sufficient calcium for enzyme stability. However, for optimal stability the final working buffer for tissue dissociation should have at least 0.1 mM Ca^{2+}.

Once completely in solution, the collagenase ***must*** be combined with a neutral protease and diluted to the appropriate volume for use in a specific tissue dissociation procedure. The collagenase may be degraded by neutral protease. To minimize this problem, the enzymes should be mixed just prior to beginning the digestion. At most, the mixture can be stored for 2 hours between 2°C and 6°C prior to use. This enzyme solution can be sterile filtered through 0.2 µm cellulose acetate or PES filter membranes without compromising enzyme potency. Surfactant free cellulose acetate (SFCA) and PES filters from several major vendors were tested and no measurable loss of CDA was observed.

Application

CI*zyme*™ Collagenase HA was developed to isolate islets from human, rat and canine pancreas. The 2,000 Wünsch unit pack size contains sufficient collagenase activity to dissociate an average size human pancreas. CI*zyme*™ Collagenase and Thermolysin (e.g., the neutral protease) have been shown to successfully isolate rodent or human islets[1]. However, a recent report suggests that *C. histolyticum* Neutral Protease (CHNP) is superior to thermolysin in yield and quality of islets recovered from human organs[2]. Refer to the website (www.vitacyte.com) or contact technical support for the latest recommendations or to discuss a specific project or objective.

CI*zyme*™ Collagenase HA is for research use only. Guidance for use of reagents in clinical cell transplantation procedures is governed by local Institutional Review Boards and regional Health Authorities.

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This product is manufactured in accordance with the principles for clinical trial material outlined in ICH Q7a[3]. The document control system in place is in alignment with FDA guidance for Phase I material. Document controls are in place to minimize the chances of cross-contamination.

Activity Assessment

VitaCyte relies on several biochemical tools to characterize and ensure the consistency of CI*zyme*™ Collagenase HA. The Pz-peptide substrate (Wünsch Assay) has historically been used to characterize collagenase activity[4]. While this assay has advantages in terms of reproducibility and historical precedence, it also has several limitations. The Wünsch Assay is strongly biased towards C2 and is not sensitive to the different molecular forms of C1. In addition, this activity assesses the catalytic activity of the enzyme and not its ability to degrade native collagen. Degraded C2 without a collagen binding domain is as active as intact C2, potentially providing misleading information about the quality of the enzyme. The limitations of the Wünsch assay led us to develop a fluorescent microplate CDA using fluorecein isothiocyanate labeled calf skin collagen fibrils as substrate[5]. The intact molecular form of purified C1 with two collagen binding domains ($C1_{116kDa}$) has approximately 10-fold higher CDA when compared the CDA found with same amount of purified C1 containing only one collagen binding domain ($C1_{100kDa}$) or intact C2 ($C2_{114kDa}$). A recent report in the literature describes the importance of intact C1 in successful human islet isolations. CI*zyme*™ Collagenase HA is manufactured to provide superior lot to lot consistency in enzyme activity[1]. Focus on manufacture of products with consistent collagenase activity enables users to focus on other issues to improve their islet isolation protocols.

In addition to the quality of the dissociation enzymes, additional factors impact the outcome of success of human islet isolations including: the quality of the organ and experience of the islet isolation team. The team needs to assess many variables that affect islet recovery[6,7]. These include but are not limited to the characteristics of the donor, transport of the organ, the tissue dissociation procedure, islet purification procedure, and assessment and subsequent culture of the islets.

Reference List

1. Balamurugan AN, Breite AG, Anazawa T, Loganathan G, Wilhelm JJ, Papas KK, Dwulet FE, McCarthy RC, and Hering BJ (2010) Successful human islet isolation and transplantation indicating the importance of class 1 collagenase and collagen degradation activity assay. *Transplantation* 89, 954-61.

2. Balamurugan AN, Loganathan G, Anazawa T, Wilhelm JJ , Yuasa T, Radosevich DM, Papas KK, Sutherland DER, McCarthy RC and Hering BJ (2009) Improved method of human islet isolation for clinical transplantation using combination of *Clostridium histolyticum* neutral protease (Serva) and high proportion of intact C1 collagenase (VitaCyte). *Xenotransplantation* 16, 545.

3. Guidance for IndustryQ7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients. (2001) http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm129098.pdf.

4. Wünsch E and Heidrich H-G. (1963) Zur quantitativen bestimmung der kollagenase. *Hoppe-Seyler's Zeitschrift Physiologische Chemie* 333, 149-151.

5. McCarthy RC, Spurlin B, Wright MJ, Breite AG, Sturdevant LK, Dwulet CS and Dwulet FE (2008) Development and characterization of a collagen degradation assay to assess purified collagenase used in islet isolation. *Transplantation Proceedings* 40, 339-42.

6. Lakey JRT, Burridge PW, and Shapiro AMJ. (2003) Technical Aspects of Islet Preparation and Transplantation. *Transplant International* 16, 613-632.

7. Nano R, Clissi B, Melzi R, Calori G, Maffi P, Antonioli B, Marzorati S, Aldrighetti L, Freschi M, Grochowiecki T, Socci C, Secchi A, Di Carlo V, Bonifacio E and Bertuzzi F. (2005) Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. *Diabetologia* 48[5], 906-12.