1. PRODUCT DESCRIPTION

Collagenase HA GMP Grade is an aseptically filled, lyophilized mixture of 60% (w/w) purified Class I (C1) and 40% (w/w) purified Class II (C2) collagenases from *Clostridium histolyticum*. The lyophilized cake/powder consists of the blended mixture in the presence of low concentration of biological buffer salts sealed under vacuum in an amber glass vial.

2. APPLICATION

Collagenase HA is designed for a variety of cell isolation procedures including non-human primates, muscle cells, adipose derived stem cells and removal of cells from collagen matrices. Collagenase HA is a highly purified collagenase product and contains negligible quantities other proteolytic activities. The product must therefore be supplemented with sufficient neutral protease to successfully release cells from extracellular matrix.

3. STORAGE & 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.

4. PRODUCT USE

4.1. Enzyme Reconstitution

Reconstitute the lyophilized enzyme with 5 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+}.

4.2. Digestion Solution Preparation

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.

4.3. Activity Concentration Recommendations

Collagenase HA 200 Wünsch unit pack size contains about 2.5 to 3.6 million CDA units total. The follow table makes recommendations for the collagenase and neutral protease activity concentrations for several cell targets.

Cell Target	Source	Collagenase HA Activity Concentration	Neutral Protease	Neutral Protease Activity Concentration
Islets[1]	Non-human Primates	85,000 – 140,000 U/mL	BP Protease	10,000 – 15,000 U/mL
Muscle Cells	Human	56,000 – 168,000 U/mL	BP Protease	13,000 – 26,000 U/mL
Cultured Cells	Collagen Matrix	25,000 – 35,000 U/mL	BP Protease	25,000 – 30,000 U/mL

4.4. Digestion Optimization

The recommendations made in this product insert represent the best guidance available based on experiences from product development activities and observations shared by users. Individual results may vary and some optimization may be required to achieve the desired outcome. Moderate adjustments to the enzyme concentration can be made with the goal of improving islet yield or minimize cell damage leading to low viability. Other factors that can be adjusted include digestion time and the mechanical contribution by digestion chamber shaking during digestion. Contact VitaCyte to discuss specific problems or optimization strategies.

5. TROUBLESHOOTING

5.1. Many factors contribute to the successful isolation of islets from rodents and inadvertent oversight to any of these conditions may drastically reduce the yield and viability of islets. While far from a complete list, the guidance below may help identify commonly encountered problems. Contact VitaCyte if this guidance does not help resolve specific issues.

5.2. Prolonged or Incomplete Digestion may be caused by:

- Loss of enzyme potency (activity)
- Incomplete enzyme rehydration during reconstitution
- Inappropriate enzyme dilution
- Presence of enzyme inhibitors
- Low incubation temperature
- Inefficient digestion solution perfusion

5.3. Low Yield and/or Cell Viability

- Prolonged organ warm ischemia time
- Aggressive mechanical disruption
- Extended incubation time
- Incubation above 37°C
- Inappropriate enzyme dilution
- Ineffective density gradient purification

6. ADDITIONAL INFORMATION

6.1. Intended Use & Regulatory

Collagenase HA GMP Grade is for ex-vivo use only to recover cells from tissue. Guidance for use of reagents in clinical cell transplantation procedures is governed by local Institutional Review Boards and regional Health Authorities. This product is manufactured in accordance with the principles for clinical trial material outlined in ICH Q7a[2]. 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.

6.2. Animal Origin

No bovine derived animal products are used in any step of manufacturing of Collagenase HA. This product is purified from culture supernatants of *C. histolyticum* that contain porcine gelatin and pancreatic enzymes derived from US and Canadian sources.

6.3. Manufacturing Summary

Enzymes are purified from the culture supernatants results from the fermentation of native organisms. The purification processes use standard protein column chromatography and tangential flow filtration concentration and diafiltration techniques. The purification processes have been optimized to yield the highest purity attainable for each enzyme while minimizing undefined and contaminating protease activities. In particular this process ensures that a negligible amount of trypsin like activity (i.e., contaminating clostripain) is present in the final product. After characterization, the purified collagenases are sterile filtered in a qualified biosafety cabinet and aseptically dispensed into amber bottles on activity units, lyophilized, sealed under vacuum then secured and labeled. The final lyophilized product is then further characterized to confirm each batch meets established specification ranges.

6.4. Activity Assessment

VitaCyte relies on several biochemical tools to characterize and ensure the consistency of Collagenase HA. The Pz-peptide substrate (Wünsch Assay) has historically been used to characterize collagenase activity[3]. 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, the substrate assesses the catalytic activity of the enzyme and does not assess the ability of collagenases to degrade native collagen. Degraded collagenases lacking a collagen binding domain are able to cleave the Pz-peptide substrate, but are not functional in degrading native collagen. The Pz-peptide activity provides potentially misleading information about the ability of collagenase to isolation islets. The limitations of the Wünsch assay led to the development a fluorescent microplate CDA using fluorecein isothiocyanate labeled calf skin collagen fibrils as substrate[4]. The intact molecular form of purified C1 with two collagen binding domains (~116kDa) has approximately 10-fold higher CDA when compared the CDA found with same amount of purified C1 containing only one collagen binding domain (~100kDa) or intact C2 (~114kDa).

6.5. Additional Considerations

In addition to the quality of the dissociation enzymes, additional factors impact the outcome of success of cell 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. 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[5].

6.6. Resources & Support

Further details on manufacturing, quality control testing and use of products are available at www.vitacyte.com or technical support at 317-917-3457.

6.7. References

1. Qi M1, Wang Y, et al. (2014) Implementation of a simplified method of islet isolation for allogeneic islet transplantation in cynomolgus monkeys. *Pancreas* 43(2), 226-35.

2. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). (2016) Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients, Guidance for Industry. www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073497.pdf

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

4. McCarthy RC, Spurlin B, et al. (2008) Development and characterization of a collagen degradation assay to assess purified collagenase used in islet isolation. *Transplantation Proceedings* 40, 339-42.

5. McCarthy RC, Breite AG, et al. (2011) Tissue dissociation enzymes for isolating human islets for transplantation: factors to consider in setting enzyme acceptance criteria. *Transplantation* 91(2), 137-45.