1. PRODUCT DESCRIPTION

CIzyme Collagenase MA 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 collagenase mixture is supplied as a frozen liquid in the presence of low concentration of biological buffer salts sealed in an amber glass vial.

2. APPLICATION

Collagenase MA is formulated to contain a sufficient amount of collagen degradation activity (CDA) units for the isolation of islets from non-human primate pancreas[1]. Collagenase MA 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 islets from the 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. 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 maintain the frozen state during transport.

4. PRODUCT USE

4.1. Digestion Solution Preparation

The collagenase must be combined with a neutral protease and diluted to the appropriate volume for use in a specific tissue dissociation procedure. CIzyme Thermolysin (4 mg for a pancreas from average size cynomolgus monkey) is recommended for this application using the form supplied as a frozen liquid (Cat# 002-2000). 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.

4.2. 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 stem cell yield or minimize cell damage leading to low viability. Other factors that can be adjusted include digestion time and the mechanical contribution by gentle agitation 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 cells. While far from a complete list, the recommendations 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

6. ADDITIONAL INFORMATION

6.1. Intended Use & Regulatory

Collagenase MA 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. 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 MA. 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. After a thorough characterization of the purified collagenases and proteases, the individual components are blended based on activity to prepare a specific formulation. This formulation is dispensed into amber vials, lyophilized and then sealed under vacuum. 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 CIzyme Collagenase MA. 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). Collagenase MA is manufactured with both 116kDa and 100kDa molecular forms of C1 to provide the optimal collagenase activity for the isolation of primate islets.

6.5. Additional Considerations

In addition to the quality of the dissociation enzymes, additional factors impact the outcome of stromal vascular cell isolation success including: the quality of the tissue and experience of the cell isolation specialist. The team needs to assess many variables that affect cell recovery. These include but are not limited to the characteristics of the donor, transport of the tissue, the tissue dissociation procedure, cell purification procedure, and assessment and subsequent culture of cells.

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. Abouaish J, Graham M, et al. (2011) Successful isolation and transplantation of nonhuman primate islets using a novel purified enzyme blend. *Transplantation* 92(8), e40-e2.

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 R.C., 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.