1. SUMMARY

Product Name	Collagenase HA
Catalog Number	001-1050
Grade	GMP Grade
Stability (Expiry)	4 years from manufacture date
Storage (Lyophilized Cake)	-20±5°C
Storage (Reconstituted Enzyme)	-20±5°C, 1 year without addition of other protease enzymes
Reconstitution Volume (mL)	5 mL
pH	7.5 ± 0.5 (upon reconstitution in water)
Reconstitution Solutions	• RO/DI Water • Cell Culture Water • Water For Injection • HBSS Buffer • Lactated Ringers • Physiological Saline • RPMI • PBS (OK for immediate use of reconstituted enzyme, NOT for long term storage of reconstituted enzyme)
Reconstitution Time	Not less than 30 minutes
Animal Origin Materials	Bovine Free. Porcine gelatin peptone is used during the fermentation of *Clostridium histolyticum* but is largely removed by downstream processing. Negligible quantities of porcine gelatin can be found in the final product.
Shipping	Enviro Ice Packs
Questions / Comments	p. 317-917-3457 e. feedback@vitacyte.com

2. PRODUCT DESCRIPTION

Collagenase HA (HA) 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 of enzymes in the presence of a low concentration of biological buffer salts and sealed under vacuum in an amber glass bottle.

3. APPLICATION

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. HA is a highly purified collagenase product and contains negligible quantities of other proteolytic activities. The product must therefore be supplemented with sufficient neutral protease to successfully release cells from extracellular matrix.

4. STORAGE & STABILITY

This product is stable for at least four years from date of manufacture if stored unopened as a lyophilized material at -20±5°C. Internal studies have shown the reconstituted enzyme is stable as a frozen solution at -20±5°C for at least one year so 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 Enviro ice packs to keep the product cold and minimize the potential for high temperature excursions during shipment.

5. PRODUCT USE

5.1. Enzyme Reconstitution

Reconstitute the HA with 5 mL of water or buffer defined in Section 1, for not less than 30 minutes to ensure complete dissolution of the enzyme. Occasionally invert the bottle 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+}.

5.2. Digestion Solution Preparation

Once completely in solution, HA must be combined with a neutral protease and diluted to the appropriate volume for use in a specific tissue dissociation procedure. HA 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 two hours between 2-8°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.

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. For most tissue types final working concentrations of 2,500 – 5,000 CDA U/mL will be effective. However, for islet recovery from non-human primate pancreas[1], concentrations will need to be in the range of 50,000 – 100,000 CDA U/mL.

6. TROUBLESHOOTING

6.1. Many factors contribute to the successful isolation of cells from tissue and inadvertent oversight to any of these conditions may drastically reduce the yield and viability of target cell population. 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.

Issue	Cause
Prolonged or Incomplete Digestion	• Loss of enzyme potency (activity) • Incomplete enzyme rehydration during reconstitution • Enzyme concentration too low • Presence of enzyme inhibitors (ex. Chelating agents) • Low incubation temperature (less than 30°C) • Poor digestion solution perfusion into organ/tissue
Low Yield and/or Viability	• Prolonged organ/tissue warm ischemia time • Aggressive mechanical disruption • Extended incubation time • High incubation temperature (above 37°C) • Enzyme concentration too high

7. ADDITIONAL INFORMATION

7.1. Intended Use & Regulatory

HA 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. HA is manufactured in accordance with the principles for clinical trial material outlined in Guidance Document Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients published by the FDA and ICH in September 2016[2]. This product is labeled as 'GMP Grade' to indicate the quality system and manufacturing facility are consistent with requirements set forth in the above document. Guidance for the qualification and acceptance of reagents used in cell therapy applications can be found in the *USP General Chapter <1043> Ancillary Materials for Cell, Gene, and Tissue-Engineered Products* and *ISO 20399 Biotechnology — Ancillary materials present during the production of cellular therapeutic products and gene therapy products*.

7.2. Animal Origin

The only animal sourced material used in the manufacture of HA is porcine gelatin peptone during the fermentation of the *Clostridium histolyticum* organism to stimulate collagenase biosynthesis. Prior to

inoculation with the organism, the culture media is sterilized for 30 minutes at 121°C, a process shown to inactivate many viruses. The subsequent purification process largely removes culture media components. The rest of the manufacturing process is entirely animal free.

7.3. Manufacturing Summary

Enzymes are purified from the culture supernatant resulting from the fermentation of native organisms. The purification process uses standard protein column chromatography, tangential flow filtration concentration and diafiltration techniques. These processes have been optimized to yield the highest purity attainable for each enzyme while minimizing undefined and contaminating protease activities. After characterization, the purified enzymes are aseptically sterile filtered in a qualified biosafety cabinet, blended based on activity to prepare a specific formulation, then aseptically dispensed into amber bottles on activity units. The bottles are lyophilized, sealed under vacuum, then secured and labeled. The final lyophilized product is then further characterized to confirm each batch meets established specification ranges.

7.4. Activity Assessment

VitaCyte relies on several biochemical tools to characterize and ensure the consistency of 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 can 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 isolate islets. The limitations of the Wünsch assay led to the development a fluorescent microplate CDA using fluorescein 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 to the CDA found with same amount of purified C1 containing only one collagen binding domain (~100kDa) or intact C2 (~114kDa).

7.5. 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/tissue and experience of the cell 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/tissue, the cell isolation procedure, and subsequent islet culture.

7.6. 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.

7.7. 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.