1. PRODUCT DESCRIPTION

Recombinant Collagenase HI (rHI) is an animal-free, aseptically filled, lyophilized mixture of purified *C. histolyticum* Class I (rC1) and Class II (rC2) collagenases expressed in recombinant *Escherichia coli*. 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 bottle.

2. APPLICATION

rHI is formulated to contain a sufficient amount of collagenase activity from rC1 and rC2 for the isolation of islets from average sized donor human pancreata. Islets from deceased donor organs have been successfully isolated using this collagenase formulation[1]. The total activity in each bottle is sufficient to release islets in pancreata up to 100 grams and the formulation represents the midpoint of a statistically driven design of experiment approach to determine optimal ratios of C1:C2[1]. rHI 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. CIzyme BP Protease has been shown to be effective in islet isolations with rHI[1].

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. 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 20 mL of water or preferred buffer 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. The digestion solution is suitable for use in most human islet isolation protocols adapted from the Riccordi

method[2,3]. VitaCyte recommends targeting 16 Wünsch of rHI per gram of trimmed pancreas for most protocols.

4.3. 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

rHI is for *ex vivo* use only for the isolation of cells. Guidance for use of ancillary reagents in clinical cell transplantation procedures is governed by local Institutional Review Boards or regional regulatory agencies. This product is manufactured in accordance with the principles for clinical trial material outlined in ICH Q7a[4]. 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 animal derived products are used in any step of manufacturing of rHI.

6.3. Manufacturing Summary

Enzymes are purified after cell disruption and extensive clarification using standard protein column chromatography and tangential flow filtration concentration and diafiltration techniques. The clarification and 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 rHI. The Pz-peptide substrate (Wünsch Assay) has historically been used to characterize collagenase activity[5]. 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. Specific Wünsch activity of purified C1 is >50 fold lower when compared to purified C2 alone. 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[6]. 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 human islet isolations[7,8] 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[9].

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

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