1. PRODUCT DESCRIPTION

Rodent Islet (RI) is an aseptically filled, lyophilized mixture of 60% purified class I (C1) and 40% purified class II (C2) collagenase from *Clostridium histolyticum* and neutral protease from *Bacillus polymyxa*. The lyophilized cake/powder consists of the blended mixture of enzymes in the presence of an inert protein stabilizing excipient in a low concentration of biological buffer salts sealed under vacuum in an amber glass vial.

2. APPLICATION

RI is formulated to contain an optimal amount of collagen degrading activity (CDA) units and neutral protease activity (NP) units for the isolation of rodent islets using the procedure described by Gotoh, et al.[1] Specific details are presented in Section 4. RI was developed using C57BL/6 mice[2] and has been successfully used to isolate islets from many strains of mice and rats. However, some strains may require a different enzyme composition and digestion time for favorable islet release and morphology. Contact VitaCyte if you encounter trouble using RI in your protocol.

3. STORAGE & STABILITY

RI is stable for at least two 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 three months. Additional studies have shown the reconstituted enzyme mixture can be successfully frozen and thawed at least twice without apparent loss of potency as assessed by the CDA and NP assays. The product is shipped on Enviro ice packs to keep the product cold and minimize the potential for high temperature excursions.

4. PRODUCT USE

4.1. Enzyme Reconstitution

Reconstitute the lyophilized enzyme with 2 mL of water or buffer (recommend HBSS or similar non-phosphate buffer) and allow enzyme to rehydrate for 15 minutes. 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, dilute the reconstituted enzyme into the desired buffer per the table below. At most, the rehydrated enzyme can be stored for 2 hours between 2°C and 8°C prior to use or freezing. Enzymes 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.

	Mouse Pancreas	Rat Pancreas
Final enzyme solution volume from 1 bottle	30 mL	45 mL

of RI		
Volume of enzyme solution into each pancreas	2-3 mL	8-10 mL
Digestion Time	~17 minutes @ 37°C	~19 minutes @ 37°C
Islet Count after Histopaque® 1077 purification	200 – 300 C57BL6 strain	800 – 1000 (200-250 gram rat)

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 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 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 (serum, EDTA, etc.)
- Low incubation temperature
- Inefficient perfusion of digestion solution

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
- Animal strain

6. ADDITIONAL INFORMATION

6.1. Intended Use & Regulatory

RI 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. 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 RI. Collagenase 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 characterization, the purified collagenases and proteases are sterile filtered in a qualified biosafety cabinet then blended on activity to prepare a specific formulation. This formulation is aseptically dispensed into amber bottles, 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

RI is assessed by several biochemical tools to characterize and ensure the consistency of components used and the final blended and lyophilized product. Collagenase activity is characterized using both the traditional Wünsch (Pz-peptide)[3] substrate and a more recently developed fluorescent microplate functional collagen degrading activity (CDA) assays[4]. The CDA assay relies on fluorecein isothiocyanate (FITC) labeled calf skin collagen fibrils as substrate. The *B. polymyxa* neutral protease activity (NPA) is measured using a FITC labeled BSA substrate[5]. Focus on manufacture of products with consistent collagenase and neutral protease activity improves control over the cell isolation process with the goal of more consistent results.

6.5. Additional Considerations

In addition to the quality of the dissociation enzymes, additional factors impact the outcome of success of rodent islet isolations including: the quality of the organ and experience of the islet isolation specialist. Differences in the yield and quality of islets can vary greatly between strains and age of animal.

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 Gotoh M, Maki T, Satomi S, Porter J, Bonner-Weir S, O'Hara C, Monaco AP. (1987) Reproducible High Yield of Rat Islets by Stationary in vitro Digestion Following Pancreatic Ductal or Portal Venous Collagenase Injection. *Transplantation* 43(5), 725-30.

2 Stull ND, Breite A, McCarthy R, Tersey SA, Mirmira RG. (2012) Mouse islet of Langerhans isolation using a combination of purified collagenase and neutral protease. *Journal of Visualized Experiments: JoVE* 67.

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., Wright M.J., Breite A.G., Sturdevant L.K., Dwulet C.S., and Dwulet F.E. (2008) Development and characterization of a collagen degradation assay to assess purified collagenase used in islet isolation. *Transplantation Proceedings* 40, 339-42.

5 Breite AG, Dwulet FE, McCarthy RC. (2010) Tissue Dissociation Enzyme Neutral Protease Assessment. *Transplant Proceedings* 42(6), 2052-4.