1. PRODUCT DESCRIPTION

Animal Origin Free Thermolysin (AFT) is an aseptically filled, lyophilized powder of purified neutral protease from *Bacillus thermoproteolyticus*[1]. The lyophilized cake/powder consists of approximately 6 mg of neutral protease in the presence of low concentration of biological buffer salts sealed under vacuum in an amber glass vial.

2. APPLICATION

AFT is designed to be mixed with collagenase for the isolation of cells, primarily islets. The amount required is dependent on the cell type, tissue source, mass of tissue and species.

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 1-2 mL of cold water or buffer for a minimum of 5 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, AFT must be combined with collagenase and diluted to the appropriate volume for use in a specific tissue dissociation procedure. AFT can degrade collagenase over time. 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 neutral protease activity was observed. The exact concentration of collagenase and protease is dependent on the specific application. Guidance for common cell targets is available at www.vitacyte.com.

4.3. Human Islet Digestion Optimization

Six mg of purified thermolysin when combined with an acceptable dose of purified *C. histolyticum* collagenase, is sufficient to isolate islets from an average size human pancreas[2] using digestion procedures based on the Riccordi method[3]. Increasing the dose of thermolysin will lead to damage of the islets and acinar cells, often leading to lower recovery of purified islets after cell culture.

5. TROUBLESHOOTING

5.1. 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.

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

5.3. Low Yield and/or Cell Viability

- Prolonged organ/tissue warm ischemia time
- Aggressive mechanical disruption
- Extended incubation time
- Elevated incubation temperature
- Inappropriate enzyme dilution

6. ADDITIONAL INFORMATION

6.1. Intended Use & Regulatory

AFT is prepared as a 'GMP Grade' enzyme. 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[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

ATF is entirely animal origin free. No animal derived materials are used in any step of the manufacturing process.

6.3. Manufacturing Summary

AFT is further processed after upon receipt from the manufacturer. After characterization, the AFT is sterile filtered in a qualified biosafety cabinet and 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

The specific neutral protease activity of purified AFT is determined by the FITC-BSA substrate NP[5].

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/tissue and experience of the cell isolation specialist. 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 cell culture[6].

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. Matsubara H. (1970) Thermolysin. *Methods Enzymology* 19, 642-650.

2. Balamurugan AN, Breite AG, et al. (2010) Successful human islet isolation and transplantation indicating the importance of class 1 collagenase and collagen degradation activity assay. *Transplantation* 89, 954-61.

3. Ricordi C. (1992) 1892-1992 One Century of Transplantation for Diabetes. *Pancreatic Islet Cell Transplantation*. Austin R.G. Landes Co. 99-112.

4. 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

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

6. 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.