

1. SUMMARY

Product Name	Animal Free (AF) Thermolysin
Catalog Number	002-3000
Grade	GMP Grade
Stability (Expiry)	2 years from manufacture date
Storage (Lyophilized Cake)	-20±5°C
Storage (Reconstituted Enzyme)	-20±5°C, (not recommended as stability has not been empirically determined and is likely dependent upon reconstitution solution and concentration)
Reconstitution Volume (mL)	2-4 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 5 minutes
Animal Origin Materials	Animal Origin Free
Shipping	Enviro Ice Packs
Questions / Comments	p. 317-917-3457 e. feedback@vitacyte.com

2. PRODUCT DESCRIPTION

Animal Free (AF) Thermolysin 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 biological buffer salts in low concentration and sealed under vacuum in an amber glass bottle.

3. APPLICATION

AF Thermolysin is designed to be mixed with collagenase for the isolation of different cell types. The amount required is dependent on the cell type, tissue source, mass of tissue, species and protocol.

4. STORAGE & STABILITY

This product is stable for at least two years from date of manufacture if stored unopened as a lyophilized material at $-20\pm 5^{\circ}\text{C}$. Internal studies have shown the reconstituted enzyme is stable as a frozen solution at $-20\pm 5^{\circ}\text{C}$ for at least three months. The product is shipped on Enviro ice packs to keep the product cold and minimize the potential for high temperature excursions.

5. PRODUCT USE

5.1. Enzyme Reconstitution

Reconstitute the lyophilized enzyme with 2-4 mL of water or buffer defined in Section 1, for not less than 5 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, AF Thermolysin must be combined with collagenase and diluted in the desired buffer to the appropriate volume for use in a specific tissue dissociation procedure. AF Thermolysin 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 two hours between $2-8^{\circ}\text{C}$ 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.

5.3. Human Islet Digestion Optimization

When combined with an acceptable dose of purified *C. histolyticum* collagenase, 6 mg of purified thermolysin is sufficient to isolate islets from an average size human pancreas² using digestion procedures based on the Riccordi method³. 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.

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.

6.2. 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 digestion solution perfusion

6.3. Low Yield and/or Cell Viability

- Prolonged organ/tissue warm ischemia time
- Aggressive mechanical disruption
- Extended incubation time
- Incubation above 37°C
- Inappropriate enzyme dilution

7. ADDITIONAL INFORMATION

7.1. Intended Use & Regulatory

AF Thermolysin 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. AF Thermolysin 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 2016⁴. 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

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

7.3. Manufacturing Summary

AF Thermolysin is further processed after receipt from the manufacturer. After characterization, the AF Thermolysin is sterile filtered in a qualified biosafety cabinet then 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.

7.4. Activity Assessment

The specific neutral protease activity of purified AF Thermolysin is determined by the FITC-BSA substrate NP⁵.

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 cell culture⁶.

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 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.