1. PRODUCT DESCRIPTION

BP Protease GMP Grade (BP) is an aseptically filled, lyophilized powder of purified neutral protease from *Paenibacillus polymyxa*[1,2]. The lyophilized cake/powder consists of the neutral protease in the presence of low concentration of biological buffer salts sealed under vacuum in an amber glass vial.

2. APPLICATION

BP is designed to be mixed with collagenase for the isolation of cells including islets, hepatocytes and adipose stem cells from tissue. 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 four years from date of manufacture if stored unopened between -20 ± 5°C. Internal studies have shown the reconstituted enzyme is stable as a frozen solution between -20 ± 5°C for at least one year as long as no other protease enzymes had been added to the solution. The product can be shipped ambient, but should be stored -20 ± 5°C.

4. PRODUCT USE

4.1. Enzyme Reconstitution

Reconstitute the lyophilized enzyme with 2 mL of cold water on ice for a minimum of 15 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, BP must be combined with collagenase and diluted to the appropriate volume for use in a specific tissue dissociation procedure. BP is prone to autolysis and will degrade steadily in solution. 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°C and 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 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. 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

BP 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. BP 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. 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. Further details can be found on the VitaCyte Commitment to Quality document available upon request.

6.2. Animal Origin

No animal derived materials are used in any step of manufacturing of BP.

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 BP is sterile filtered in a qualified biosafety cabinet and aseptically dispensed into amber vials on activity units, 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 BP is determined by the FITC-BSA substrate NP[3].

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 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 culture.

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. Fogarty WH, Griffin PJ. (1973) Production and Purification of the Metalloprotease of Bacillus polymyxa. *Applied Microbiol.* 26(2), 185-190.

2. Griffin PJ, Fogarty WH. (1973) Physiochemical Properties of the Native, Zinc- and Manganese-Prepared Metalloprotease of Bacillus polymyxa. *Applied Microbiol.* 26(2), 191-195.

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