1. PRODUCT DESCRIPTION

Clostripain is an aseptically filled, lyophilized purified unactivated enzyme from *Clostidium histolyticum*. The lyophilized cake/powder consists of clostripain in the presence of low concentration of biological buffer salts under vacuum in an amber glass bottle.

2. APPLICATION

Clostriapin is used as a supplement to collagenase and other proteases in tissue dissociation applications. Each tissue type and primary cell of interest is has its own clostripain requirement. Clostripain is dispensed by mass and the total BAEE units of enzyme activities are detailed on the lot specific certificate of analysis. The requirements for use of clostripain are confounded by the fact that as a cysteine protease, the oxidized form of clostripain is far less active than the reduced form. Treatment with a reducing agent will convert the unactivated form into the active form which is approximately 5-10 fold higher in specific activity[1]. However, there are special considerations that need to be taken into account if using reduced clostripain. Contact VitaCyte to discuss specific details on how to work with clostripain in your application.

3. STORAGE & STABILITY

The product is stable for at least two years from date of manufacture if stored between -15 to -25°C. Internal real time stability data indicates the enzyme is stable in excess of 5 years as a lyophilized powder. The reconstituted enzyme can be stored for up to 3 months as a frozen liquid.

4. PRODUCT USE

4.1. Enzyme Reconstitution

The product is supplied as a lyophilized cake in vacuum sealed amber bottles. Reconstitute the lyophilized enzyme with 2-5 mL of cold water or desired buffer on ice for a minimum of 20 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. Failure to allow the enzyme to completely rehydrate will affect the enzyme potency and could negatively impact the success of the tissue dissociation procedure.

4.2. Digestion Solution Preparation

Reconstituted clostripain must be combined with an appropriate amount of purified collagenase and diluted to the final volume in desired buffer for use in a digestion procedure. The collagenase-clostripain mixture should be stored for less than 2 hours at 2 to 8°C prior to use. Clostripain is known to be detrimental to collagenase enzymes and should be mixed immediately before the dissociation procedure. This enzyme solution can be sterile filtered through 0.2 µm cellulose acetate or PES filter membranes without compromising enzyme potency.

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.

5. TROUBLESHOOTING

Three factors generally contribute to the success of tissue dissociation: the quality of the organ or tissue, the quality of enzymes and consistency with previous lots used successfully, and experience of the isolation team. The team needs to assess many variables that affect the outcome of the digestions. These include but are not limited to the characteristics of the donor, transport of the organ, the tissue dissociation procedure, cell purification procedures, and assessment and subsequent culture of cells.

6. ADDITIONAL INFORMATION

6.1. Intended Use & Regulatory

Clostripain 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[2]. 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 Collagenase HA. This product 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

Clostripain is 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 a thorough characterization of the purified protease, the enzyme is sterile filtered, aseptically 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

The peptide substrate N-benzoyl-L-arginine ethyl ester (BAEE) is the traditional method of assaying clostripain activity. This peptide was originally developed to detect trypsin activity, but works for clostripain

as well. The assay is run in the buffer described in the reference for the unactivated activity and also in the presence of a reducing agent (DTT) to determine the activated clostripain activity[1,3].

6.5. 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. REFERENCES

1) Mitchell WM and Harrington WF. (1970) *Methods in Enzymology* 19:635-42.
2) 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
3) Van Wart H.E. and Steinbrink D.R. (1981) A continuous spectrophotometric assay for *Clostridium histolyticum* collagenase. *Analytical Biochemistry* 113, 356-365.