	VitaCyte, LLC	Version: 1
	Product Insert	Date: 28 Sept 2023
Clostripain		Cat# 004-2000

1. SUMMARY


Product Name	Clostripain
Catalog Number	004-2000
Grade	GMP Grade
Stability (Expiry)	2 years from manufacture date
Storage (Lyophilized Cake)	-20±5°C
Storage (Reconstituted Enzyme)	-20±5°C, 3 months without addition of other protease enzymes
Reconstitution Volume (mL)	2-5 mL
Reconstitution Solutions	<ul style="list-style-type: none"> • 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	No less than 20 minutes
Animal Origin Statement	Bovine Free. Porcine gelatin peptone is used during the fermentation of <i>Clostridium histolyticum</i> but is largely removed by downstream processing. Negligible quantities of porcine gelatin can be found in the final product.
Shipping	Enviro Ice Packs
Questions/ Comments	Call VitaCyte at 317-917-3457 OR Email VitaCyte at feedback@vitacyte.com

2. PRODUCT DESCRIPTION

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

3. APPLICATION

Clostripain is used as a supplement to collagenase-protease mixtures in tissue dissociation applications. Each tissue type and primary cell of interest has its own clostripain requirement. Clostripain is dispensed by mass

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and the total unactivated BAEE units (TLA or native activity) and total activated BAEE units (Clostripain activity) per bottle are reported 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 (e.g. DTT, Cysteine) will convert the unactivated form into the active form which is approximately 5-10 fold higher in specific activity¹. However, there are special concerns that need to be considered if using reduced clostripain. Contact VitaCyte to discuss specific details on how to work with clostripain in your application.

4. STORAGE & STABILITY

The 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 real time stability data indicates the enzyme is stable in excess of five years as a lyophilized powder. Internal studies have also shown the reconstituted enzyme is stable as a frozen solution at $-20\pm 5^{\circ}\text{C}$ for up to three months provided that repeated freezing and thawing are avoided. 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

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 bottle 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. Avoid buffers that contain chelating agents such as EDTA since clostripain is calcium-dependent.

5.2. Digestion Solution Preparation

Reconstituted clostripain must be combined with an appropriate purified collagenase (Collagenase HA, Collagenase MA, DE 100) and protease (BP Protease, Thermolysin) mixture then further diluted with desired buffer to the final volume required for the respective digestion procedure. Collagenase-protease-clostripain mixtures should be constantly stored on ice after blending together. Clostripain is known to be detrimental to collagenase enzymes so enzyme blends should be used within two hours of combining if properly stored at 2 to 8°C. Otherwise, enzyme working solutions should be prepared immediately before the tissue dissociation procedure. This enzyme solution can be sterile filtered through 0.2 µm cellulose acetate or PES filter membranes without compromising enzyme potency.

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5.3. 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. For most tissue digestion protocols, 0.25 to 0.50 unactivated BAEE U/mL are recommended. For isolation of islets from a human pancreas, 1.0 to 1.5 unactivated BAEE U/g tissue are recommended.

6. 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.

7. ADDITIONAL INFORMATION

7.1. Intended Use & Regulatory


Clostripain 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. This product is manufactured in accordance with the principles for clinical trial material outlined in ICH Q7a². 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.

7.2. Animal Origin

No bovine derived animal products are used in any step of manufacturing of clostripain. This product is purified from culture supernatants of *C. histolyticum* that contain porcine gelatin and pancreatic enzymes derived from US and Canadian sources.

7.3. Manufacturing Summary

Clostripain is purified from the culture supernatant resulting from the fermentation of native organisms. The purification process uses standard protein column chromatography, tangential flow filtration concentration and diafiltration techniques. The purification processes have been optimized to yield the highest purity attainable for the enzymes while minimizing undefined and contaminating protease activities. After characterization of the purified protease, the enzyme is sterile filtered, aseptically dispensed into amber bottles, lyophilized, and then sealed under vacuum. The final lyophilized product is then further characterized to confirm each batch meets established specification ranges.

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7.4. 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 in the presence of a reducing agent (DTT) to determine the activated clostripain activity^{1,3}.

7.5. 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.

7.6. 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.