1. PRODUCT DESCRIPTION

DE Collagenases are a product line of aseptically filled, lyophilized preparation of enriched (85 – 90% pure) *Clostridium histolyticum* collagenases and purified neutral protease from *Bacillus polymyxa*. The enzymes are formulated in a minimally hygroscopic, polypeptide excipient to maintain enzyme stability during storage and convenience of weighing precise amounts of enzyme. The DE Collagenase product line includes 5 formulations of collagenases and protease in which the quantity of protease is constant across all 5 products but an increasing quantity of collagenase between each formulation. This provides a range of collagenase to neutral protease ratios as a practical approach to optimizing tissue dissociation and subsequent cell recovery protocols. Each formulation is available in both a 100 mg and 1 g gram pack size.

DE Collagenase	Pack Size	Catalog #	Target Collagenase	Target Protease	Ratio (FALGPA/SCU)
DE10/100	100 mg/1 g	011-1110/011-1010	10/100 FALGPA Units	1.8/18 mg	0.022
DE20/200	100 mg/1 g	011-1120/011-1020	20/200 FALGPA Units	1.8/18 mg	0.044
DE40/400	100 mg/1 g	011-1130/011-1030	40/400 FALGPA Units	1.8/18 mg	0.089
DE60/600	100 mg/1 g	011-1140/011-1040	60/600 FALGPA Units	1.8/18 mg	0.133
DE80/800	100 mg/1 g	011-1150/011-1050	80/800 FALGPA Units	1.8/18 mg	0.178

2. APPLICATION

The required activity of the DE Collageanses to recover cells will vary significantly depending on the tissue source and protocol used. Contact VitaCyte for technical guidance on how to evaluate the use of DE Collagenases in specific applications. However, a concentration range of 0.5 – 1.5 mg/mL is a realistic starting point to evaluate cell recovery on many tissue types.

3. STORAGE & STABILITY

DE Collagenase is stable for at least two years from date of manufacture if stored as a lyophilized powder at ≤ 2-8°C.

4. PRODUCT USE – Enzyme Reconstitution

While preparing for tissue digestion, equilibrate DE Collagenase to room temperature. DE Collagenase is supplied as a lyophilized powder. In some cases this powder may appear is a solid cake or in clumps when first received. Vigorous shaking of the bottle or mechanical disruption with a laboratory spatula should quickly convert the material into a partially flowing powder. Weigh out the required amount of enzyme powder. Remaining enzyme may be resealed in the bottle returned to storage at 2-8°C.

The weighed out enzyme needs to be rehydrated. Collagenase Gold may be reconstituted in a small volume of buffer or water and further diluted into the working buffer (suggest HBSS or a similar non-phosphate buffer) or added directly to the desired volume of working buffer. Once the enzyme has been added to solution, allow the powder to rehydrate for a minimum of 15 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. 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 Ca2+ and contain no cation chelating agents. The 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 CDA was observed.

5. ADDITIONAL INFORMATION

5.1. Intended Use & Regulatory

DE Collagenases are 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. 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.

5.2. Animal Origin

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

5.3. 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. After characterization, the purified collagenases are sterile filtered in a qualified biosafety cabinet and aseptically dispensed into amber bottles on activity units, lyophilized, then secured and labeled. The final lyophilized product is then further characterized to confirm each batch meets established specification ranges.

5.4. Activity Assessment

Each lot of product is characterized for collagenase activity using the FALGPA peptide substrate[1] and neutral protease activity using succinyl casein substrate[2]. The clostripain and trypsin-like activities are determined on the specific lot of enriched collagenase used to prepare Collagenase Gold products[3]. The amount of these activities is calculated based on the amount of collagenase dispensed into each product.

5.5. Resources & Support

VitaCyte has details on the significance of enzyme activities and the assays used in the manufacturing and quality control of products on the website, www.vitacyte.com. Additional details about enzyme activity or questions regarding your specific isolation are available by contacting VitaCyte directly. Additional reagents can be ordered by sending an e-mail to orders@vitacyte.com or by phone at (317) 917-3457.

6. REFERENCES

1. Van Wart HE and Steinbrink DR. *Analytical Biochemistry* 113 (1981); 356-65.
2. Hatakeyma T, Kohzaki H, and Yamasaki N. *Analytical Biochemistry* 204 (1992); 181-184.
3. Mitchell WM and Harrington WF. *Methods in Enzymology* 19 (1970) 635-642.