VitaCyte® Unravelling Cell Isolation	VitaCyte, LLC	Version: 3
	Product Insert	Date: 15 May 2019
Collagenase Gold		Cat# 011-1060 (1 g) Cat# 011-1160 (100 mg)

1. PRODUCT DESCRIPTION

Collagenase Gold is an aseptically filled, lyophilized preparation of enriched (85 – 90% pure) *Clostridium histolyticum* collagenases formulated in a minimally hygroscopic, polypeptide excipient to maintain enzyme stability during storage and convenience of weighing precise amounts of enzyme. Collagenase Gold is sold as 100 mg (Cat# 011-1160) or 1 g (Cat# 011-1060) pack sizes where the mass represents the dry weight of powder.

2. APPLICATION

Collagenase Gold is prepared with a minimum of either 80 (100 mg pack size) or 800 (1 g pack size) FALGPA units per vial[1]. The required activity 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 Collagenase Gold 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. Collagenase Gold contains minimal quantity of neutral protease activity and in the majority cases will need to be supplemented with a neutral protease for successful cell recovery.

3. STORAGE & STABILITY

Collagenase Gold is stable for at least four years from date of manufacture if stored as a lyophilized powder at ≤ 2-8°C. The product can be shipped ambient, but should be stored ≤ 2-8°C.

4. PRODUCT USE

4.1. Enzyme Reconstitution

While preparing for tissue digestion, equilibrate Collagenase Gold to room temperature. Collagenase Gold is supplied as a lyophilized powder. In some cases this powder may appear as a solid cake or in clumps when first received. Vigorous shaking of the vial 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 vial and 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 vial 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 Ca^{2+} 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

Collagenase Gold 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. 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 Collagenase Gold. 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 vials 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]. The clostripain and trypsin-like activities are determined on the specific lot of enriched collagenase used to prepare Collagenase Gold products[2]. The amount of these activities is calculated based on the amount of collagenase dispensed into each product.

5.5. 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.

5.6. References

1. Van Wart HE and Steinbrink DR. *Analytical Biochemistry* 113 (1981); 356-65.

2. Mitchell WM and Harrington WF. *Methods in Enzymology* 19 (1970) 635-642.