1. SUMMARY

Product Name	Collagenase Gold
Catalog Number	011-1060
Grade	For Research Use Only
Stability (Expiry)	4 years from manufacture date
Storage (Lyophilized Cake)	≤ 2-8°C
Storage (Reconstituted Enzyme)	-20±5°C, 1 year without addition of other protease enzymes
Reconstitution Volume (mL)	Dependent on mass
Reconstitution Solutions	• 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	Not less than 15 minutes
Animal Origin Statement	Bovine Free. Porcine gelatin peptone is used during the fermentation of *Clostridium histolyticum* but is largely removed by downstream processing. Non-mammalian gelatin peptone used in the final manufacturing of the final product.
Shipping	Ambient
Questions / Comments	p. 317-917-3457 e. feedback@vitacyte.com

2. PRODUCT DESCRIPTION

Collagenase Gold is an aseptically filled, lyophilized preparation of enriched (85 – 90% pure) *Clostridium histolyticum* collagenase formulated in a minimally hygroscopic, polypeptide excipient to maintain enzyme stability during storage and provide convenience of weighing precise amounts of enzyme. The product contain minimal amounts of neutral protease activity. and sold as ≈ 1 g pack size where the mass represents the dry weight of powder.

3. APPLICATION

Collagenase Gold is prepared with a minimum of 800 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. For many enzyme mediated cell isolation applications, the collagenase solution must be supplemented with neutral protease activity. A concentration range of 0.5 – 1.5 mg/mL is a realistic starting point to evaluate cell recovery on many tissue types.

The Collagenase Gold product can be used with PD Collagenase 800 (011-3020) to prepare purified enzyme mixtures to replace your existing product. To use this method, you will need to estimate the mass of collagenase used in the enzyme solution you prepare for cell isolation. See Appendix 1 for further details of the Rational Design Method for Tissue Dissociation Enzyme (RDMTDE) optimization.

4. STORAGE & STABILITY

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

5. PRODUCT USE

5.1. 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 water (RO/DI, Cell Culture, Water for Injection), buffer (HBSS, Physiological Saline, PBS, Lactated Ringers) or media (RPMI), and further diluted into the working 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. 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.

6. ADDITIONAL INFORMATION

6.1. 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 aligned 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 Collagenase Gold. Collagenase 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

Enzymes are purified from the culture supernatant resulting from the fermentation of native organisms. The purification processes use standard protein column chromatography, 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.

6.4. 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.

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.

6.6. 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.

Appendix 1 The Rational Design Method for Tissue Dissociation Enzyme (RDMTDE) optimization

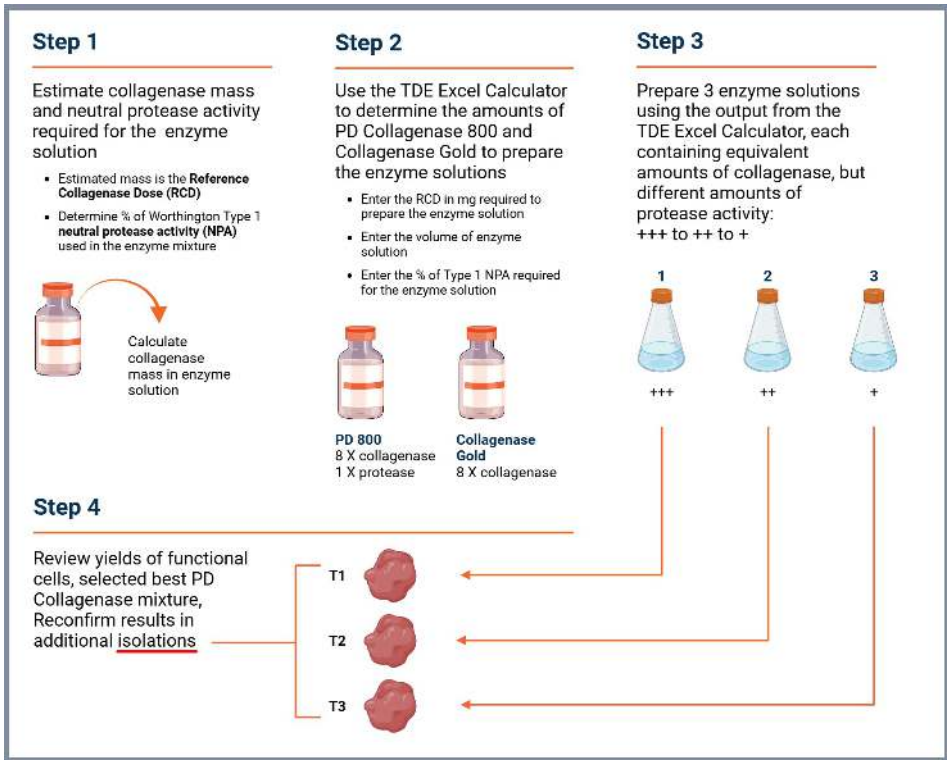
The RDMTDE method uses the PD Collagenase 800 and Collagenase Gold (001-1060) products. The method applies the principles described in a mechanistic model of enzyme-mediated cell isolation[4,5] to optimize collagenase and protease activities used in this process. A 2 minute video of this model can be viewed on VitaCyte's website. The key elements of the model are summarized below.

- The extracellular matrix (ECM) is a jungle of proteins where collagen fibrils/fibers predominate
- Collagen shields other ECM proteins from proteolytic degradation
- Cells are tethered to tissue by cell anchoring proteins within the extracellular matrix (ECM) which are directly or indirectly associated with collagen fibrils or fibers
- Proteases alone are unable to free cells from tissue, enzyme mixtures must contain a sufficient amount of collagenase's collagen degradation activity to degrade the collagen substrate
- *Clostridium histolyticum* collagenases thin the collagen jungle and disrupts the ECM
- Collagenase degrades collagen by the synergistic attack of class I (C1) and class II (C2) collagenase on individual tropocollagen molecules:
    - C1 is a processive protease that shaves of the collagen by moving along the molecule from the carboxy to the amino terminus
    - C2 is an endo protease that makes internal cuts on the collagen
- Clearing of collagen by collagenase leads to exposure of protease-sensitive sites on the ECM proteins
- Proteolytic cleavage of these exposed sites leads to proteases breaking the molecular tethers that hold cells to the ECM, leading to release of individual cells or cellular aggregates from tissue.

Three important principles are derived from this model:

- Collagenase's collagen degradation activity (CDA) must be in excess to ensure thinning and loosening of the extracellular matrix
- If CDA is in excess, then neutral protease activity controls the speed of tissue digestion and yield of functional, viable cells
- Neutral protease activity required for successful cell isolation is equivalent or less than the neutral protease activity found in Worthington Type 1 Collagenase

The RDMTDE described below applies these three principles to simplify the generation of a purified collagenase-protease enzyme mixture for your cell isolation procedure in 3 steps as described in the infographic below.



Note PD Collagenase 800 and Collagenase Gold contain the same amount of collagenase. Only PD Collagenase 800 contains neutral protease activity. This activity is close to the activity found in a 1 g bottle of Worthington Collagenase Type 1. The broad application of Type 1 Collagenase to digest any mammalian tissue means that referencing the enzyme mixture to the percentage of neutral protease activity found in Type 1 Collagenase serves as a useful reference point.

Step:1

Estimate the mg of collagenase and the neutral protease activity required for the enzyme solution. This amount is referred to as the **Reference Collagenase Dose (RCD)** . If you do not know the neutral protease activity, assume that 100% of the Worthington Collagenase Type 1 activity is sufficient for cell isolation. It is useful to prepare several enzyme solutions with decreasing neutral protease activities to assess its effect on functional cell yield and viability.

Step 2:

Use the TDE Excel Calculator to determine the amounts of PD Collagenase 800 and Collagenase Gold to prepare the enzyme solutions. Enter the mg of collagenase, mL of enzyme solution, and percentage of Worthington Collagenase Type 1 neutral protease activity required to prepare each enzyme solution. The TDE Excel calculator can be downloaded from VitaCyte's website.

Step 3: Determine if VitaCyte's products provide comparable results to those obtained using your current collagenase enzyme. You may find slower digestion times as the neutral protease activity is decreased as would be expected from the hypothetical model of enzyme-mediated cell isolation. You will need to determine if extending the digestion time provides other benefits to the recovered cell population.

Reviewing Results

If the results are comparable to those obtained with your current lot of product, **congratulations!** You now know more about the enzyme formulation required for isolating your cell of interest

If you do not obtain equivalent results with any of the enzyme mixtures above when compared to your current lot of collagenase, this may indicate

- The collagenase is inappropriate, add more collagenase
- Add another, supplemental protease to increase the degradation of ECM proteins

Clostripain is a common contaminant of purified collagenase preparations. It may be required to achieve comparable cell isolation results. It is a trypsin-like enzyme that cuts at different regions of the protein than BP Protease. Review VitaCyte's Clostripain Package Insert for more details or contact VitaCyte Technical Support.

The effort required to assess the performance of the three enzyme solutions above will not change your effort to qualify new lots of traditional crude or enriched collagenase products. The benefits of adopting the PD Collagenase enzyme in place of traditional collagenase are summarized in the table below.

	Lot Qualification	PD Optimization
Effort required	Equivalent	Equivalent
Knowledge gained	None, no knowledge of enzyme composition	Essential: enzyme composition defined
Lot pre-qualification	No change, must prequalify new lots	Once defined, no need to prequalify future lots
Ability to modify formulation	None	Yes
Shelf stability fo product	?	4 years

If you have any questions about using enzymes for cell isolation or recovery, contact VitaCyte Technical Support.