1. SUMMARY

Product Name	PD Collagenase 100 (PD 100), PD Collagenase 800 (PD 800)
Catalog Number	011-3010/011-3020
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 USE

2.1. Enzyme Reconstitution

While preparing for tissue digestion, equilibrate PD Collagenase to room temperature. PD Collagenase is supplied as a lyophilized powder. This powder may appear as a solid cake or 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. The remaining enzyme may be resealed in the bottle and returned to storage at 2-8°C.

PD Collagenase 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. 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. Enzyme denaturation may occur if the enzyme solution is vortexed or swirled excessively. 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 collagenase's collagen degradation activity was observed. See details for preparing a mixture of the PD Collagenase 100 and 800 products in Appendix 1.

2.2. Digestion Solution Preparation

See Appendices 1 & 2 for details on how to use the product.

3. PRODUCT DESCRIPTION

PD Collagenase 100 and 800 products are aseptically filled, lyophilized preparations of > 95% pure *Clostridium histolyticum* collagenases and purified neutral protease from *Paenibacillus polymyxa* with a composition detailed in the table below. These two products are equivalent to the DE Collagenase 100 and 800 products sold earlier by VitaCyte.

Components	1g PD Collagenase 100	1g PD Collagenase 800
C. histolyticum collagenase	55 mg	440 mg
BP Protease (Dispase™ equivalent enzyme)	18 mg	18 mg
Approximate mg polypeptide excipient	927 mg	542 mg

The minimally hygroscopic polypeptide excipient preserves enzyme stability during storage and adds convenience because users can weigh the precise amount of product needed immediately before use. These lyophilized products showed no loss of enzyme activity after stressing the lyophilized products by incubation at higher temperatures. These products have a five-year shelf life at 4°C.

The PD 100 product is formulated to have enzyme activities like those found in Worthington Type 1 collagenase, the first crude collagenase product used for cell isolation. The PD 800 product contains an eight-fold increase in collagenase mass. This formulation was defined by reverse engineering "good lots" of crude collagenase used for human islet isolation. The high collagenase-low protease activity mixture works

well for many other cell types. This product contains the same amount of BP Protease found in the PD 100 product.

4. APPLICATION

- The PD Collagenase 100 and 800 alone or mixed in different ratios enable you to develop a continuum of collagenase to protease ratios for application-specific formulations.
- If the DE Collagenase product line was used in the past, see Appendix 1 to determine how to use the PD 100 and 800 products to prepare the enzyme formulations equivalent to DE 200, 400, or 600 products.
- Alternatively, the PD 800 product can be used with Collagenase Gold (001-1060) 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 2 for further details of the Rational Design Method for Tissue Dissociation Enzyme (RDMTDE) optimization.

5. STORAGE & STABILITY

PD Collagenase is stable for at least five years from the date of manufacture if stored as a lyophilized powder at ≤ 2-8°C. The product is shipped ambient but should be stored ≤ 2-8°C. Internal studies have shown the reconstituted enzyme is stable as a frozen solution at -20±5°C for at least one year as long as no other protease enzymes had been added to the solution.

6. TROUBLESHOOTING

6.1. Many factors contribute to the successful isolation of cells from tissue and inadvertent oversight to any of these conditions may drastically reduce the yield and viability of target cell population. While far from a complete list, the guidance below may help identify commonly encountered problems. Contact VitaCyte if this guidance does not help resolve specific issues.

6.2. Prolonged or Incomplete Digestion may be caused by:

- Loss of enzyme potency (activity)
- Incomplete enzyme rehydration during reconstitution
- Inappropriate enzyme dilution
- Presence of enzyme inhibitors
- Low incubation temperature
- Inefficient digestion solution perfusion

6.3. Low Yield and/or Cell Viability

- Prolonged organ warm ischemia time
- Aggressive mechanical disruption
- Extended incubation time
- Elevated incubation temperature
- Inappropriate enzyme dilution

7. ADDITIONAL INFORMATION

7.1. Intended Use & Regulatory

PD Collagenases are for research use only.

7.2. Animal Origin

No bovine-derived animal products are used in any step of manufacturing PD Collagenases. Collagenase is purified from culture supernatants of C. histolyticum that contain porcine gelatin and pancreatic enzymes. Before lyophilization, a non-mammalian peptide excipient is added to the solution containing the purified enzyme mixture.

7.3. Manufacturing Summary

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 by volume into amber bottles to contain 1 g dry weight of protein product. The final lyophilized product is then further characterized to confirm each batch meets established specification ranges.

7.4. 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 collagenase used to prepare PD Collagenase products[3].

7.5. Additional Considerations

In addition to the quality of the dissociation enzymes, additional factors impact the outcome of success of cell isolations including: the quality of the organ/tissue and experience of the cell isolation team. The team needs to assess many variables that affect islet recovery. These include but are not limited to the characteristics of the donor, transport of the organ/tissue, the cell isolation procedure, and subsequent cell culture.

7.6. 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.

7.7. References

1 Van Wart HE and Steinbrink DR. A continuous spectrophotometric assay for Clostridium histolyticum collagenase. Analytical Biochemistry 113 (1981); 356-65

2 Hatakeyma T, Kohzaki H, and Yamasaki N. A micro assay for proteases using succinylcasein as a substrate. Analytical Biochemistry 204 (1992); 181-184.

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

4 McCarthy RC, Breite AG, Green ML, Dwulet FE. Tissue dissociation enzymes for isolating human islets for transplantation: factors to consider in setting enzyme acceptance criteria. Transplantation 91 (2011) 137-45.

5 McCarthy RC, Green ML, Dwulet FE. Evolution of enzyme requirements for human islet isolation. OBM Transplantation 2 (2018) 024

8. APPENDICIES

-Appendix 1

Refer to the table below to prepare products equivalent to the former DE Collagenase 200, 400, or 600 using PD Collagenase 100 & 800 products.

Enzyme Formulation in reference to PD 100 product (Collagenase/Protease activity ratio)	**Directions to prepare 1 mg equivalents of PD 200, PD 400, or PD 600 using PD 100 and PD-800**	
	PD-Collagenase 100 (Cat # 011-3010)	PD Collagenase 800 (Cat # 011-3020)
DE 200 (2.6)	85.7% = 6 parts PD 100	14.3% = 1 part PD 800
DE 400 (5.2)	57.1% = 4 parts PD 100	42.9% = 3 parts PD 800
DE 600 (7.8)	28.6% = 2 parts PD 100	71.4% = 5 parts PD 800

The illustrative table shows how to prepare 100 mg of DE 200, DE 400, or DE 600

To check your calculations, add the mg of the PD 100 and PD 800. The answer should be the amount of DE 200, DE 400, or DE 600 products required for your cell isolation procedure.

Prepare the enzyme mixtures by weighing the appropriate mass of each product shortly before use—reconstitute and use within two hours. DO NOT STORE POWDERED MIXTURES OF THE TWO PRODUCTS FOR RE-USE SINCE THE HOMOGENEITY OF THE NEW MIXTURE IS NOT KNOWN.

	100 mg
DE 200	100 x 0.857 = 86 mg PD 100 + 100 x 0.143 = 14 mg PD 800
DE 400	100 x 0.571 = 57 mg PD 100 + 100 x 0.429 = 43 mg PD 800
DE 600	100 x 0.286 = 29 mg PD 100 + 100 x 0.714 = 71 mg PD 800

Appendix 2 The Rational Design Method for Tissue Dissociation Enzyme (RDMTDE) optimization

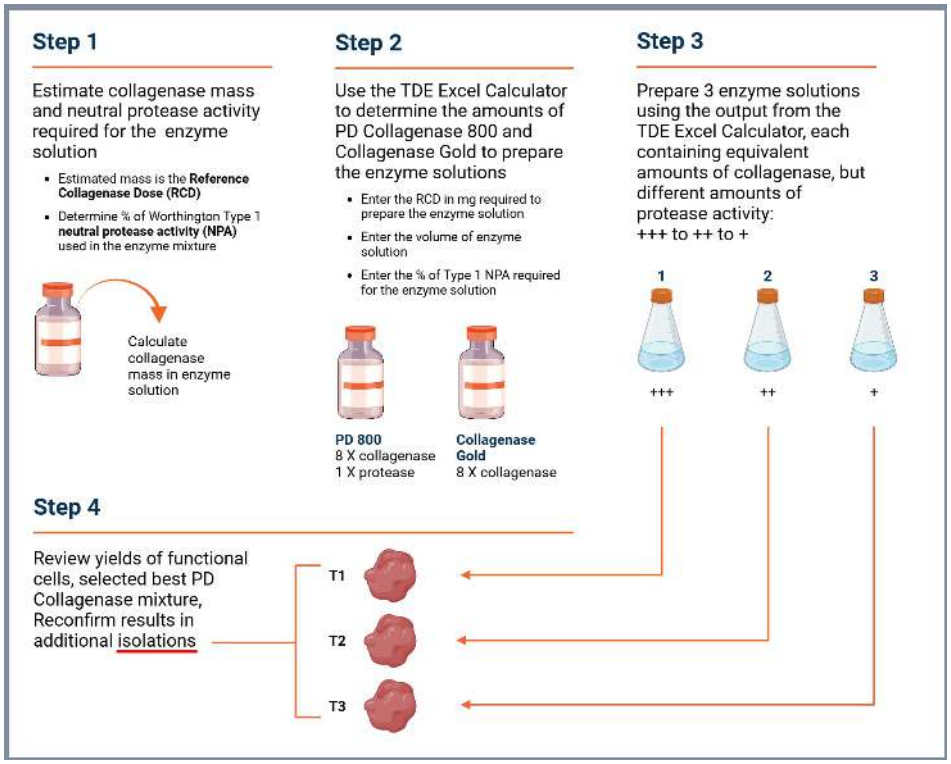
The RDMTDE method uses the PD Collagenase 800 and Collagenase Gold (001-1060) products. The method applies the principles described in a mechanistic model of enzyme-mediated cell isolation[4,5] to optimize collagenase and protease activities used in this process. A 2 minute video of this model can be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