



ProteoExtract[®] Collagenase Set

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About the Kit

Description

The ProteoExtract[®] Collagenase Set includes crude, lyophilized preparations of Type I, II, III, and IV *Clostridium histolyticum* that are qualified for dissociation of mammalian tissues.

Components

- 100 mg Collagenase Type 1, lyophilized powder, ≥ 125 units per mg dry weight
- 100 mg Collagenase Type 2, lyophilized powder, ≥ 125 units per mg dry weight
- 100 mg Collagenase Type 3, lyophilized powder, ≥ 100 units per mg dry weight
- 100 mg Collagenase Type 4, lyophilized powder, ≥ 160 units per mg dry weight

Storage

Store lyophilized collagenases at 2–8°C.

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Overview

The matrix that holds cells together in animal tissue is a complex mixture of proteins, glycoproteins, lipids, glycolipids, and mucopolysaccharides. To recover individual cells, this matrix must be effectively broken down without causing extensive damage to the cells. Each of the four crude preparations of collagenase includes a mixture of proteolytic activities that selectively digest the intercellular matrix while limiting damage to the cells.

The ProteoExtract® Collagenase Set includes crude preparations of Type I, II, III, and IV *Clostridium histolyticum*. Each of the four collagenases is qualified for tissue dissociation. This kit is ideal for use with the ProteoExtract Tissue Dissociation Buffer Set (Cat. No. 539720) and when developing an optimal dissociation protocol for varied types of tissue. The kit includes 100 mg of each lyophilized enzyme.

Collagenase	Description	Activity	Storage
Type I	Generally recommended for adipocytes, epithelial, hepatocytes, lung, and adrenal cell isolation. Contains average amounts of collagenase, caseinase, clostripain and trypsin.	≥ 125 units* per mg dry weight	4° C
Type II	Generally used for cardiomyocytes, bone, cartilage, muscle, thyroid, and endothelial cell isolation. Contains higher protease contaminant levels, especially clostripain and trypsin.	≥ 125 units per mg dry weight	4° C
Type III	Typically used for mammary and other soft tissues. Contains lower levels of contaminating proteases.	≥ 100 units per mg dry weight	4° C
Type IV	Typically used for pancreatic islets and other applications where membrane protein and receptor integrity is crucial. Contains especially low trypsin activity.	≥ 160 units per mg dry weight	4° C

*One unit of collagenase activity releases one micromole of L-leucine equivalents from native bovine achilles tendon collagen at 37°C in 5 hours (Moore and Stein, 1948).

For optimal results, the type of collagenase preparation must be determined empirically for the tissue to be dissociated. The above recommendations are based on results from several different research groups. Additionally, the amount of collagenase necessary to dissociate a tissue is dependent on the tissue type and must be determined empirically. See 'Considerations Before You Begin' (p 3) for general guidelines.

Considerations Before You Begin

- The ProteoExtract[®] Tissue Dissociation Buffer Kit (Cat. No. 539720) provides an optimized buffer set and detailed protocol for collagenase digestion of animal tissues.
- The collagenases can be resuspended in most balanced salt solutions (BSS), including phosphate-buffered saline (PBS), Hanks' (HBSS), and Earl's (EBSS).
- The pH optimum for the collagenases is 7–9.
- Collagenases are activated by calcium and zinc and are inhibited by metal chelating agents including cysteine, EDTA, EGTA, o-phenanthroline, thiol compounds, and difolate.
- We recommend preparing fresh solutions of each collagenase immediately prior to use. If necessary, the reconstituted collagenases should be stored in small aliquots at –20 or –80°C. Avoid freeze/thaw cycles. Do not store the reconstituted collagenases at 4°C.
- We recommend using approximately 10,000 units of collagenase per gram of tissue as a starting point. Note, however, that the amount of collagenase necessary to fully digest a tissue will depend on several factors including but not limited to tissue type, collagenase type, digestion buffer, time, and temperature.
- We recommend using the collagenases based on a measure of their units of activity and not weight. Crude preparations of collagenase can differ significantly in the number of units per mg of dry weight, resulting in up to a two-fold difference in the amount of enzymatic activity added to the sample.

Collagenase Digestion of Animal Tissues

The following protocol should be used only as a guideline for dissociation of animal tissues when using the ProteoExtract Collagenase Set. Details of the dissociation protocol should be empirically determined for each tissue type and collagenase.

Note: The ProteoExtract[®] Tissue Dissociation Buffer Kit (Cat. No. 539720) includes optimized buffers for use with the Collagenase Set.

1. Cut 1 g fresh tissue into small pieces (e.g., ~10–20 pieces per g tissue) using a scalpel or scissors.
2. Wash the minced tissue 2 times in PBS or other suitable buffer using gentle agitation in a petri dish.
3. Transfer the minced tissue to 15 ml conical tube.
4. Add approximately 10 ml of a balanced salt solution containing an appropriate amount of collagenase and incubate for 50 min at 37°C with gentle agitation (e.g. using a hybridization oven).

Note: As a starting point, we recommend using 10,000 units of collagenase per g of tissue.

5. Following incubation, strain the sample using a sieve and discard the undissociated tissue fragments.
6. Transfer the dissociated tissue (i.e. the material that passes through the sieve) to a fresh tube and add approximately 15 ml of an appropriate balanced salt solution containing protease inhibitors.
7. Centrifuge for 5 min at 350 g and 4°C to pellet the dissociated tissue.
8. Resuspend the pellet in approximately 15 ml of an appropriate balanced salt solution containing protease inhibitors and centrifuge as in step 7.
9. Resuspend the pellet in approximately 15 ml of an appropriate balanced salt solution containing protease inhibitors.
10. Determine the number and viability of the cells resulting from the tissue dissociation step. A standard hemocytometer is recommended for determining the number of cells, and trypan blue stain for determining their viability.

References

1. Moore, S. and Stein, W.H. (1948) *J. Biol. Chem.* **176**:367.