

Manufactured for:



## Collagenase

Cat. No.:	17100	Type I
	17101	Type II
	17102	Type III
	17103	Hepatocyte Qualified
	17104	Type IV

Sizes: See catalog.

*Custom pack sizes available upon request.*

Storage: 2 to 8°C (-5 to -20°C after reconstitution)

Avoid moisture and exposure to light.

Avoid inhalation and skin contact.

This product is intended for cell or tissue disaggregation only.

### Background

Collagenase (from clostridium histolyticum) is a protease with a specificity for the bond between a neutral amino acid (x) and glycine in the sequence Pro-X-Glyc-Pro. This sequence is found in high frequency in collagen and is unique among proteases in its ability to degrade the triplehelical native collagen fibrils commonly found in connective tissue.

The collagenase most commonly used for tissue dissociation is a crude preparation containing clostripolypeptidase A and a number of other proteases, polysaccharidases and lipases. This crude enzyme is ideally suited for tissue dissociation since it contains the enzyme required to attack native collagen and reticular fibers, in addition to the enzymes which hydrolyze the other proteins, polysaccharides and lipids in the extracellular matrix of connective and epithelial tissues.

Crude collagenase does exhibit lot-to-lot variability and may produce occasional toxicity. Invitrogen attempted to minimize these difficulties by tissue-typing their crude collagenase lots based upon a correlation between various enzyme levels in each preparation and effectiveness in dissociating certain tissues.

### Specifications

**Potency:** One unit liberates 1 µM of L-leucine equivalents from collagen in 5 hours at +37°C, pH 7.5.

**Types:** Particular enzymatic activities of crude collagenases have correlated with the tissues from which the cells were obtained (or with the uses to which the cells are put), and as a result of the correlation's, several formal types have been established.

**Type III** Selected because of low proteolytic activity. (casein as substrate)

**Type IV** Selected because of low tryptic activity. (BAEE as substrate)

These selected types have been found to give better performance in preparation of cells from the various tissues as tabulated below. It should be noted, however, that while the results have been greatly improved following this classification, there is still some lot-to-lot variation, and efforts continue in attempting to gain even better control over crude collagenase.

TYPE	TISSUES OR CELLS
I	Fat cells, Adrenal, Liver
II	Heart, Bone, Thyroid, Cartilage, Liver
III	Mammary
IV	Islet (insulin receptor sites)

**Inhibitors:** Metal chelating agents such as cysteine, EDTA or o-phenanthroline but not DFP. It is also inhibited by  $\alpha_2$ -macroglobulin, a large plasma glycoprotein.

### Instructions for use

#### A. Preparing stock and working solution

Dissolve the non-sterile, lyophilized enzyme in HBSS (Cat. No. 14025). Filter sterilize the solution with a cell culture approved filtration unit. Crude collagenase is most often used in concentrations from 0.1 to 0.5% (W/V) or 50 to 100 U/mL. Once reconstituted use immediately or store frozen. Thaw in refrigerator immediately prior to use.

#### B. Dissociation of tissue

- Tissue is minced with a sterile scalpel or scissors.
- Wash the tissue several times in HBSS.
- The tissue fragments are soaked at +37°C. Increased efficiency is obtained using a rocker platform and supplementing the digest with 3 mM CaCl<sub>2</sub>.

#### C. Organ perfusion

- Digest is prewarmed to +37°C and perfused at a rate preoptimized for the particular organ. Addition of 3 mM CaCl<sub>2</sub> increases the efficiency of dissociation.
- Dispersed cells and tissue fragments are separated from larger pieces by passing the mixture through a sterile stainless steel or nylon mesh. Fresh collagenase solution can be added to the fragments if further disaggregation is required.
- Wash several times to eliminate debris and enzyme solution. A density separation step (Nycodenz) will give a cleaner suspension.
- Resuspend the pellet in the culture medium and incubate under predetermined conditions.

For further information on this or other GIBCO™ products, contact Technical Services at the following:

United States TECH-LINE<sup>SM</sup>: 1 800 955 6288

Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at [www.invitrogen.com](http://www.invitrogen.com).

**For research use only.**

**CAUTION: Not intended for human or animal diagnostic or therapeutic uses.**

June 2004

Form No. 3743