



## COLLAGENASE

### *Tissue Dissociation/Cell Isolation*

The first commercially available collagenase isolated from *Clostridium histolyticum* was offered by Worthington in 1959. At that time we offered one type of crude enzyme which we only tested for collagenase activity. Eventually, with the cooperation of many in the research community, four basic enzyme profiles were identified:

**Type 1** contains average amounts of assayed activities; collagenase, caseinase, clostripain and trypsin. It is generally recommended for adipocytes, epithelial, hepatocytes, lung and adrenal cell isolation.

**Type 2** contains higher protease contaminant levels, especially clostripain and trypsin. It is generally used for cardiomyocytes, bone, cartilage, muscle, thyroid and endothelial cell isolation.

**Type 3** contains lower levels of contaminating proteases. It is typically used for mammary and other soft tissues.

**Type 4** contains especially low trypsin activity. It is typically used for pancreatic islets and other applications where membrane protein and receptor integrity is crucial.

**CLSPA**, Chromatographically purified collagenase, is specially purified to reduce the caseinase activity and provide high specific activity. It is typically used for pancreatic and parotid acini isolation and collagen structural analysis.

Worthington also offers 0.22 $\mu$ m filtered collagenase preparations of each Type in 50 mg per vial pre-packaged form for direct reconstitution and use in cell isolation and culture procedures. Correlations between enzyme type and effectiveness with different tissues have been good, but not perfect, due in part to the variable parameters of use. Nevertheless most researchers consider the tissue-typing of crude collagenase lots to be a valuable service. A detailed description of the Worthington collagenase and contaminant assays can be found in the **Worthington Enzyme Manual**. In addition tissue specific references and detailed isolation conditions can be found in the **Worthington Tissue Dissociation Guide**. Please request your copy or go to [www.worthington-biochem.com](http://www.worthington-biochem.com) or [www.tissuedissociation.com](http://www.tissuedissociation.com).

<u>Product</u>	<u>Activity</u>	<u>Code</u>	<u>Cat. No.</u>	<u>Size</u>	<u>Price</u>
<b>Chromatographically Purified.</b> Contains less than 50 caseinase units per milligram. Composed of two separable but very similar collagenases. Supplied as a lyophilized powder. Store at 2-8°C.	$\geq$ 300 units per mg dry weight	CLSPA	LS005275	4,000 U	\$ 70.00
			LS005273	10,000 U	145.00
			LS005277	Bulk	Inquire
<b>Type 1</b> containing average amounts of assayed activities; collagenase, caseinase, clostripain and trypsin. It is generally recommended for adipocytes, epithelial, hepatocytes, lung and adrenal cell isolation. Store at 2-8°C.	$\geq$ 125 units per mg dry weight	CLS 1	LS004194	100 mg	\$ 30.00
			LS004196	1 gm	156.00
			LS004197	5 gm	665.00
			LS004200	Bulk	Inquire
<b>0.22<math>\mu</math>m Filtered CLS 1.</b> Lyophilized, 50 mg per vial. Store at 2-8°C.	$\geq$ 125 units per mg dry weight	CLSS 1	LS004214	1 vial	\$ 36.00
			LS004216	5 vials	132.00
<b>Type 2</b> contains higher protease contaminant levels, especially clostripain and trypsin. It is generally used for cardiomyocytes, bone, cartilage, muscle, thyroid and endothelial cell isolation. Store at 2-8°C.	$\geq$ 125 units per mg dry weight	CLS 2	LS004174	100 mg	\$ 30.00
			LS004176	1 gm	156.00
			LS004177	5 gm	665.00
			LS004179	Bulk	Inquire

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<u>Product</u>	<u>Activity</u>	<u>Code</u>	<u>Cat. No.</u>	<u>Size</u>	<u>Price</u>
<b>0.22µm Filtered CLS 2.</b> Lyophilized, 50 mg per vial. Store at 2-8°C.	≥125 units per mg dry weight	CLSS 2	LS004202 LS004205	1 vial 5 vials	\$ 36.00 132.00
<b>Type 3</b> contains lower levels of contaminating proteases. It is typically used for mammary and other soft tissues. Store at 2-8°C.	≥100 units per mg dry weight	CLS 3	LS004180 LS004182 LS004183 LS004185	100 mg 1 gm 5 gm Bulk	\$ 30.00 156.00 665.00 Inquire
<b>0.22µm Filtered CLS 3.</b> Lyophilized, 50 mg per vial. Store at 2-8°C.	≥100 units per mg dry weight	CLSS 3	LS004206 LS004208	1 vial 5 vials	\$ 36.00 132.00
<b>Type 4</b> contains especially low trypsin activity. It is typically used for pancreatic islets and other applications where membrane protein and receptor integrity is crucial. Store at 2-8°C.	≥160 units per mg dry weight	CLS 4	LS004186 LS004188 LS004189 LS004191	100 mg 1 gm 5 gm Bulk	\$ 30.00 156.00 665.00 Inquire
<b>0.22µm Filtered CLS 4.</b> Lyophilized, 50 mg per vial. Store at 2-8°C.	≥160 units per mg dry weight	CLSS 4	LS004210 LS004212	1 vial 5 vials	\$ 36.00 132.00

Connective tissue develops from mesenchymal cells and forms the dermis of skin, the capsules and stroma of several organs, the sheaths of neural and muscular cells and bundles, mucous and serous membranes, cartilage, bone, tendons, ligaments and adipose tissue.

Connective tissue is composed of cells and extracellular fibers embedded in an amorphous ground substance and is classified as loose or dense, depending upon the relative abundance of the fibers. The cells, which may be either fixed or wandering, include fibroblasts, adipocytes, lymphocytes, monocytes, eosinophils, neutrophils, macrophages, mast cells and mesenchymal cells.

Collagen fibers are present in varying concentrations in virtually all connective tissues. Measuring 1-10 µm in thickness, they are unbranched and often wavy, and contain repeating transverse bands at regular intervals. Biochemically, native collagen is a major fibrous component of animal extracellular connective tissue; skin, tendon, blood vessels, bone, etc. In brief, collagen consists of fibrils composed of laterally aggregated polarized tropocollagen molecules (M.W. 300,000). Each rod-like tropocollagen unit consists of three helical polypeptide α-chains wound around a single axis. The strands have repetitive glycine residues at every third position and an abundance of proline and hydroxyproline. The amino acid sequence is characteristic of the tissue of origin. Tropocollagen units combine uniformly in a lateral arrangement reflecting charged and uncharged amino acids along the molecule, thus creating an axially repeating periodicity. Fibroblasts and possibly other mesenchymal cells synthesize the tropocollagen subunits and release them into the extracellular matrix where they undergo enzymatic processing and aggregation into native collagen fibers. Interchain cross-linking of hydroxyprolyl residues stabilizes the collagen complex and makes it more insoluble and resistant to hydrolytic attack by most proteases. The abundance of collagen fibers and the degree of cross-linking tend to increase with advancing age, making cell isolation more difficult.

Reticular fibers form a delicate branching network in loose

connective tissue. They exhibit a regular, repeating subunit structure similar to collagen and may be a morphological variant of the typical collagen fibers described above. Reticular fibers tend to be more prevalent in tissues of younger animals.

Bacterial collagenase is a crude complex containing a collagenase more accurately referred to as clostridiopeptidase A which is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most frequently a neutral amino acid. Such sequences are often found in collagen, but only rarely in other proteins. While many proteases can hydrolyze single-stranded, denatured collagen polypeptides, clostridiopeptidase A is unique among proteases in its ability to attack and degrade the triple-helical native collagen fibrils commonly found in connective tissue.

True collagenase may cleave simultaneously across all three chains or attack at a single strand. Mammalian collagenases split collagen in its native triple-helical conformation at a specific site yielding fragments, TC A and TC B, representing 3/4 and 1/4 lengths of the tropocollagen molecule. After fragmentation the pieces tend to uncoil into random polypeptides and are more susceptible to attack by other proteases.

Bacterial collagenases are usually extracted from host invasive strains. These enzymes differ from mammalian collagenases in that they attack many sites along the helix. Collagenases from *Clostridium histolyticum*, first prepared by Mandl, *et al.*, have been most thoroughly studied. Commercially available collagenase has been limited primarily to that from *Cl. histolyticum*; although, other sources have recently become available. Clostridial collagenase also degrades the helical regions in native collagen preferentially at the X-Gly bond in the sequence Pro-X-Gly-Pro where X is most frequently a neutral amino acid. This bond in synthetic peptide substrates may also be split.

Purified clostridiopeptidase A alone is usually inefficient in dissociating tissues due to incomplete hydrolysis of all

collagenous polypeptides and its limited activity against the high concentrations of non-collagen proteins and other macromolecules found in the extracellular matrix. The collagenase most commonly used for tissue dissociation is a crude preparation containing clostridiopeptidase A in addition to a number of other proteases, polysaccharidases and lipases. Crude collagenase is well 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.

## Applications

Most applications for primary cell isolation, culture and tissue dissociation utilize crude collagenase to dissociate individual cells from animal tissues. Collagenase is better suited than trypsin for these applications because the intercellular matrix contains large amounts of collagen. The use of trypsin alone produces very slow dissociation since the collagen remains intact. Incubation with trypsin for times long enough to dissociate cells can cause extensive damage to cells and a loss of viability.

As detailed the matrix holding cells together in animal tissue is a complex mixture of proteins, glycoproteins, lipids, glycolipids and mucopolysaccharides. For isolation of cells or establishing primary cultures this matrix must be effectively broken down with minimal alteration to cell surfaces and intracellular structures. Treatment of tissues with crude collagenase with its mixture of proteolytic activities provides gentle, selective digestion of the intercellular matrix with little damage to cells or loss of viability.

For best results the precise mixture of collagenase and proteolytic activities must be tailored to the tissue to be dissociated. Worthington's four different types of crude collagenase offer these different mixtures of activities and are recommended for specific tissue types based upon evaluation by numerous researchers. In addition these proteolytic contaminant levels can vary from lot-to-lot making lot sampling critical to certain applications. For these reasons Worthington offers the **Collagenase Sampling Program** detailed separately and at [www.worthington-biochem.com](http://www.worthington-biochem.com) and [www.tissuedissociation.com](http://www.tissuedissociation.com).

Collagenase is typically used at concentrations from 0.05 % to 0.5 % (w/v) in balanced salt solutions such as Hank's, Earle's and others.

It is important to note that historically most cell isolation protocols have cited the enzyme concentration used in terms of weight per unit volume (w/v). More recently, however, some researchers have begun to use the enzymes on an activity basis, that is, units per unit volume (u/v). Either method can be used however the advantages and disadvantages of each should be considered:

- The traditional weight per unit volume method most likely resulted from the use of cruder, partially purified mixtures of enzymes and is used independently of any specific or contaminating activities which may be present. With some of these crude preparations the lot-to-lot variation can be significant resulting in up to a two-fold difference in the amount of enzymatic activity added on a weight basis.
- Use of the enzyme on an activity basis can conversely result in a two-fold difference in the amount of weight added to a dissociation; however, this normalizes the potency used based upon the primary activity for each lot.

Both methods ignore the relative contaminant activity levels. Upon establishing a basic method consider pre-sampling different lots of enzyme(s) to evaluate these factors and to select a lot of enzyme which has minimal effect upon the critical parameters of a specific application.

## Collagenase Sampling Program

Providing researchers with the best combination of cell yield and viability is the aim of the Worthington Collagenase Sampling Program. The lot-to-lot variation which is typical of crude collagenase makes it important to pre-test a particular lot in many applications. Over the years we have found that the most practical approach for the researcher is to pre-sample several different lots at one time and select the best of the group. As the world's leading manufacturer of collagenase Worthington is able to offer the greatest number of different lots at any given time and can recommend specific lots for an application.

Under the program Worthington provides individual researchers with 100 mg samples of up to three different lots of collagenase **free of charge**, for evaluation in their own application. A period of 60 days is allowed for your evaluation during which a minimum of 3 grams of each lot of collagenase will be placed on HOLD, reserved in your name. When you determine which lot performs best for you simply specify the lot desired when ordering. The only requirement is that, when and if a suitable lot of collagenase is found, a minimum of 3 grams be purchased. There is no cost or obligation for participating in the collagenase sampling program.

To become part of this program, or to discuss any of the many Worthington products, just call our Technical Service group toll-free at **800.445.9603**, Fax: **800.368.3108** from anywhere in the continental United States or Canada. Samples can also be requested on-line at [www.worthington-biochem.com](http://www.worthington-biochem.com) and [www.tissuedissociation.com](http://www.tissuedissociation.com) under the Collagenase Sampling Program header.

## ***New Collagenase Lot Selection Tool Now Available Online!***

Worthington's "***Collagenase Lot Selection Tool***" is now available online at our website. This new feature was designed to help researchers select and evaluate current collagenase lots that match previous lots or desired activity profiles. Users may enter target values for collagenase, caseinase, clostripain, and tryptic activities or specify previous lot numbers. Each value can be weighted based upon the relative level of importance to the application. After the search for matches is completed, a ranked list of collagenase lots currently available is generated.

The selected lots can then be sampled simply by using the built-in link to the ***Free Collagenase Sampling Program***. As always, Worthington Customer and Technical Service personnel are available via phone at 800.445.9603 / 732.942.1660 and e-mail to assist with collagenase or any other products.

## Stability

All Worthington collagenases are supplied as lyophilized powders and are stable for at least 1-2 years when stored at 2-8°C. Long term stability can be extended by storage at -20°C; however, frequent freeze-thaw cycles and moisture absorption should be avoided.

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## Activity

All Worthington collagenases are assayed using the following methods:

Collagenase activity is measured using a modification of the collagen digestion procedure of Mandl, *et al.* in which the enzyme is incubated for 5 hours with native bovine achilles tendon collagen (WBC Code: CL) at 37°C. The extent of collagen digestion is determined using the Moore and Stein colorimetric ninhydrin method. The amino acids released are expressed as micromoles leucine per milligram dry weight of collagenase. *One unit equals one micromole of L-leucine equivalents released from collagen in 5 hours at 37°C, pH 7.5, under the specified conditions.*

Caseinase activity, a measure of non-specific proteolytic activity, is determined using the above assay and substituting 25 milligrams vitamin free casein for the collagen substrate. This reaction is stopped after 5 hours by the addition of 0.5 ml of 50% trichloroacetic acid (TCA). After centrifugation 0.2 ml of the supernatant is transferred to 1 ml of ninhydrin and handled as above. Caseinase activity is calculated as for collagenase activity.

Clostripain activity is measured after activation in 2.5 mM dithiothreitol (DTT). *One unit hydrolyzes one micromole of BAEE per minute at 25°C, pH 7.6, after activation.*

Tryptic activity is assayed using the same BAEE method as clostripain, but without activation.

Activators of collagenase include calcium and zinc. Inhibitors include metal chelating agents such as cysteine, EDTA, EGTA, o-phenanthroline, thiol compounds and difolate.

### **Related Products**

**Elastases  
Hyaluronidase  
Papains  
Trypsins  
Cell Isolation Optimizing System  
Hepatocyte Isolation System  
Neonatal Cardiomyocyte Isolation Kit  
Papain (Neural) Dissociation System**

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**Complete Catalog, Tissue Dissociation  
Guide and Enzyme Manual available  
on-line at:**

**[www.worthington-biochem.com](http://www.worthington-biochem.com)**  
**[www.tissuedissociation.com](http://www.tissuedissociation.com)**