

*Clostridium histolyticum* contains two distinct but related genes for collagenase. The *col G* gene codes for a 936 amino acid protein designated Collagenase Type 1 and the *col H* gene codes for a 1021 amino acid protein designated Collagenase Type II. Partially purified collagenase preparations contain several isoforms of both these gene products, a sulfhydryl protease, clostripain, a trypsin-like enzyme, and an aminopeptidase. This combination of collagenolytic and proteolytic activities is effective at breaking down intercellular matrices, the essential part of tissue dissociation. One component of the complex is a hydrolytic enzyme that degrades the helical regions in native collagen preferentially at the Y-Gly bond in the sequence Pro-Y-Gly-Pro, where Y is most frequently a neutral amino acid. This cleavage yields products susceptible to further peptidase digestion. Partially purified collagenase is inhibited by 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.  $Ca^{2+}$  is required for enzyme activity. Particular enzymatic profiles of each collagenase have been correlated with the tissues from which the cells for study were obtained (or with the uses to which the cells are put). As a result of the correlations, several types of partially purified collagenases have been established by Worthington: **Types 1, 2, 3, 4, 5, 6 and 7.**



- **Type 1** partially purified collagenase has the original balance of collagenase, caseinase, clostripain and tryptic activities.
- **Type 2** contains higher relative levels of protease activity, particularly clostripain.
- **Type 3** contains lowest levels of secondary proteases.
- **Type 4** is designed to be especially low in tryptic activity to limit damage to membrane proteins and receptors.
- **Type 5** contains higher collagenase and caseinase values.
- **Type 6** contains high collagenase activity with a caseinase to collagenase ratio ~2:1. Designed to be enriched for Type II (*col H*) collagenase relative to Type I (*col G*).
- **Type 7** contains collagenase and caseinase activities four-fold higher than collagenase Types 1 and 2.
- Purified collagenase, Codes: **CLSPA/CLSPANK**, contain minimal secondary proteolytic activities along with high collagenase activity.









Animal Free Types AFA, AFB, AFC, AFD, AFP, STZ1 and STZ2 collagenases are derived from cultures grown in medium completely devoid of animal based components and designed for bioprocessing applications where introduction of potential animal derived pathogens must be prevented. Levels of secondary proteases are similar to Types 1 and 2 collagenase.

- **CLSAFA** is the original AF grade designed to have collagenase and secondary proteases similar to Types 1 and 2 collagenase.
- **CLSAFB** contains higher collagenase and caseinase activities than CLSAFA.
- **CLSAFC** has especially low tryptic activity similar to Type 4 collagenase.
- **CLSAFD** contains two to three fold higher specific activity than CLSAFA.
- Purified collagenase, Code: **CLSAFP** contains minimal secondary proteolytic activities along with high collagenase activity.
- **STZ1 & STZ2**, 0.22 $\mu$  filtered **STEMxyme**<sup>®</sup> AF Collagenase/Neutral Protease (Dispase<sup>®</sup>) blends for primary and stem cell isolation.

Worthington also offers 0.22 micron filtered preparations of many types in 50 mg/vial pre-packaged form for direct reconstitution and use in all isolation 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 partially purified 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 [Worthington-Biochem.com](http://Worthington-Biochem.com) or [TissueDissociation.com](http://TissueDissociation.com).

Description	Activity	Code	Catalog No.	Size
<b>Collagenase, Purified</b> Chromatographically purified. $\leq 50$ caseinase units per milligram. Supplied as a lyophilized powder. Store at 2-8°C.	$\geq 500$ units per mg dry weight	<b>CLSPA</b>	LS005275 LS005273 LS005277	4 ku 10 ku Bulk
<b>Collagenase Vial, NCIS</b> A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of HBSS or equivalent yields a solution of 300 units/ml of collagenase, Code: CLSPA. Suitable for cell isolation and culture applications. Store at 2-8°C.	$\geq 1,500$ units per vial	<b>CLSPANK</b>	LK003240 LK003245	1 vi 5 vi
<b>Collagenase, Type 1</b> The original balance of enzymatic activities. Each lot assayed for collagenase, caseinase, clostripain and tryptic activities. Suggested for epithelial, liver, lung and adrenal primary cell isolations. A dialyzed, lyophilized powder. Store at 2-8°C.	$\geq 125$ units per mg dry weight	<b>CLS-1</b>	LS004194 LS004196 LS004197 LS004200	100 mg 1 gm 5 gm Bulk
<b>Collagenase, Type 2</b> Prepared to contain higher clostripain activity. Suggested for bone, heart, liver, thyroid and salivary primary cell isolation. Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	$\geq 125$ units per mg dry weight	<b>CLS-2</b>	LS004174 LS004176 LS004177 LS004179	100 mg 1 gm 5 gm Bulk
<b>Collagenase, Type 3</b> Lower in secondary proteolytic contaminant activities but with typical collagenase activity. Suggested for mammary primary cell isolation. A dialyzed, lyophilized powder. Store at 2-8°C.	$\geq 100$ units per mg dry weight	<b>CLS-3</b>	LS004180 LS004182 LS004183 LS004185	100 mg 1 gm 5 gm Bulk
<b>Collagenase, Type 4</b> Prepared to contain lower tryptic activity levels to limit damage to membrane proteins and receptors but with normal to above normal collagenase activity. Suggested for pancreatic islet primary isolation. A dialyzed, lyophilized powder. Store at 2-8°C.	$\geq 160$ units per mg dry weight	<b>CLS-4</b>	LS004186 LS004188 LS004189 LS004191	100 mg 1 gm 5 gm Bulk
<b>Collagenase, Type 5</b> Prepared to contain higher collagenase and caseinase activities. A dialyzed, lyophilized powder. Store at 2-8°C.	$\geq 450$ units per mg dry weight	<b>CLS-5</b>	LS005280 LS005282 LS005283 LS005284	100 mg 1 gm 5 gm Bulk
<b>Collagenase, Type 6</b> Prepared to contain high collagenase activity with a caseinase to collagenase ratio $\sim 2:1$ . Designed to be enriched for Type II (col H) collagenase relative to Type I (col G). A dialyzed, lyophilized powder. Store at 2-8°C.	$\geq 400$ units per mg dry weight	<b>CLS-6</b>	LS005318 LS005319 LS005321 LS005323	100 mg 500 mg 2.5 gm Bulk
<b>Collagenase, Type 7</b> Prepared to contain collagenase and caseinase activities four-fold higher than collagenase Types 1 and 2. A dialyzed, lyophilized powder. Store at 2-8°C.	$\geq 1,000$ units per mg dry weight	<b>CLS-7</b>	LS005332 LS005333 LS005335 LS005337	100 mg 500 mg 2.5 gm Bulk

Description	Activity	Code	Catalog No.	Size
<b>Collagenase, Type 1, Filtered</b> Collagenase, Type 1 (Code:CLS-1), which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.	≥125 units per mg dry weight	<b>CLSS-1</b>	LS004214 LS004216 LS004217	50 mg 5 x 50 mg 1 gm
<b>Collagenase, Type 2, Filtered</b> Collagenase, Type 2 (Code:CLS-2), which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.	≥125 units per mg dry weight	<b>CLSS-2</b>	LS004202 LS004204 LS004205	50 mg 5 x 50 mg 1 gm
<b>Collagenase, Type 3, Filtered</b> Collagenase, Type 3 (Code:CLS-3), which is filtered through a 0.22 micron membrane and lyophilized in vials to contain ≥ 50 milligrams per vial. Store at 2-8°C.	≥100 units per mg dry weight	<b>CLSS-3</b>	LS004206 LS004208	50 mg 5 x 50 mg
<b>Collagenase, Type 4, Filtered</b> Collagenase, Type 4 (Code:CLS-4), which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.	≥160 units per mg dry weight	<b>CLSS-4</b>	LS004210 LS004212 LS004209	50 mg 5 x 50 mg 1 gm
<b>Collagenase, Type 5, Filtered</b> Collagenase, Type 5 (Code:CLS-5), which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.	≥450 units per mg dry weight	<b>CLSS-5</b>	LS005286 LS005287 LS005288	50 mg 5 x 50 mg 1 gm
<b>Collagenase/Elastase Vial, HIS Kit</b> Worthington collagenase (Code:CLS-1) and elastase (Code:ESL), filtered through 0.22 µm pore size membrane, and lyophilized. A component of the HIS kit also contains 30 u/vial elastase. Store unconstituted vials at 2-8°C.	≥20,000 units per vial	<b>CLSH</b>	LK002066 LK002067	1 vi 5 vi
<b>STEMxyme®1, Collagenase/ Neutral Protease, 0.22 Filtered</b> A specialized combination of Animal Free <i>Clostridium histolyticum</i> collagenase and Animal Free <i>Bacillus polymyxa</i> neutral protease with a minimum of 250 CLS units and 1,000 caseinase units per mg dry weight. Designed for stem cell and other primary cell isolations and bioprocessing applications where introduction of potential animal derived pathogens must be prevented. Store at 2-8°C.	≥250 collagenase units per mg dry weight ≥1,000 caseinase units per mg dry weight	<b>STZ1</b> 	LS004106 LS004107	50 mg 5 x 50 mg
<b>STEMxyme®2, Collagenase/ Neutral Protease, 0.22 Filtered</b> A specialized combination of Animal Free <i>Clostridium histolyticum</i> collagenase and Animal Free <i>Bacillus polymyxa</i> neutral protease with a minimum of 250 CLS units and 2,000 caseinase units per mg dry weight. Designed for stem cell and other primary cell isolations and bioprocessing applications where introduction of potential animal derived pathogens must be prevented. Store at 2-8°C.	≥250 collagenase units per mg dry weight ≥2,000 caseinase units per mg dry weight	<b>STZ2</b> 	LS004112 LS004113	50 mg 5 x 50 mg

Description	Activity	Code	Catalog No.	Size
<p><b>Collagenase, Purified</b> Prepared from cultures grown in medium completely devoid of animal based components and designed for bioprocessing applications where introduction of animal derived pathogens must be prevented. Chromatographically purified. <math>\leq 50</math> caseinase units per milligram. Supplied as a lyophilized powder. Store at 2-8°C.</p>	$\geq 1,500$ units per mg dry weight	<p><b>CLSAFP</b></p> 	<p>LS005290 LS005292 LS005294</p>	<p>4 ku 10 ku Bulk</p>
<p><b>Collagenase, Type A</b> Collagenase derived from cultures grown in animal-free medium. Store at 2-8°C.</p>	$\geq 150$ units per mg dry weight	<p><b>CLSAFA</b></p> 	<p>LS004152 LS004154 LS004156 LS004158</p>	<p>100 mg 1 gm 5 gm Bulk</p>
<p><b>Collagenase, Type A, 0.22 Filtered</b> Collagenase, Animal Free Type A which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.</p>	$\geq 150$ units per mg dry weight	<p><b>CLSAFAS</b></p> 	<p>LS004118 LS004119</p>	<p>50 mg 5 x 50 mg</p>
<p><b>Collagenase, Type B</b> Prepared from cultures grown in medium completely devoid of animal based components Contains higher collagenase and caseinase activities than CLSAFA. Store at 2-8°C.</p>	$\geq 300$ units per mg dry weight	<p><b>CLSafb</b></p> 	<p>LS004145 LS004147 LS004148 LS004150</p>	<p>100 mg 1 gm 5 gm Bulk</p>
<p><b>Collagenase, Type B, 0.22 Filtered</b> Collagenase, Animal Free Type B which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.</p>	$\geq 300$ units per mg dry weight	<p><b>CLSAFBS</b></p> 	<p>LS004124 LS004125</p>	<p>50 mg 5 x 50 mg</p>
<p><b>Collagenase, Type C</b> Prepared from cultures grown in medium completely devoid of animal based components. Low tryptic activity similar to Type 4. Store at 2-8°C.</p>	$\geq 200$ units per mg dry weight	<p><b>CLSAFC</b></p> 	<p>LS004138 LS004140 LS004141 LS004143</p>	<p>100 mg 1 gm 5 gm Bulk</p>
<p><b>Collagenase, Type C, 0.22 Filtered</b> Collagenase, Animal Free Type C which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.</p>	$\geq 200$ units per mg dry weight	<p><b>CLSAFCS</b></p> 	<p>LS004130 LS004131</p>	<p>50 mg 5 x 50 mg</p>
<p><b>Collagenase, Type D</b> Prepared from cultures grown in medium completely devoid of animal based components. Specific activity two to three fold higher than CLSAFA. Store at 2-8°C.</p>	$\geq 600$ units per mg dry weight	<p><b>CLSAFD</b></p> 	<p>LS004160 LS004162 LS004163 LS004165</p>	<p>100 mg 500 mg 2500 mg Bulk</p>

### Related Products

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## Applications

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. For best results the precise mixture of collagenase and proteolytic activities must be tailored to the tissue to be dissociated. Worthington's different types of partially purified 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 Worthington-Biochem.com and TissueDissociation.com.

## 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. **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. 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.

## 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 partially purified collagenase makes it important to pre-test a particular lot in many applications. 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.

## Collagenase Lot Selection Tool Available Online

Worthington's Collagenase Lot Selection Tool is available online at our website. This 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, clostripan, 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**.

**Go to: <https://www.worthington-biochem.com/support/collagenase-sampling-program>**

**For current citations in real-time, go to the online product listings and reference the Bioz Stars in the yellow highlighted area:**

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## Worthington Collagenase Products, Specifications and Applications Table

Product Code	Collagenase	Caseinase	Clostripain	Tryptic	Comments/Applications*
	CDU/mgdw	u/mgdw	u/mgdw	u/mgdw	
<b>Partially Purified</b>					
CLS-1	≥125	≥200	≤4.0	≤0.5	Balanced activities/Adipose, Adrenal, Epithelial, Liver, Lung
CLS-2	≥125	≥200	≥3.5	≥0.1	Higher proteolytic activities/Bone, Heart, Liver, Thymus
CLS-3	≥100	≥50	≤3.0	≤0.3	Lower proteolytic activities/Mammary
CLS-4	≥160	≥100	≤3.0	≤0.1	Lower tryptic activity/Pancreatic Islets
CLS-5	≥450	≥450	≤4.0	≤0.3	Higher collagenase and caseinase activities
CLS-6	≥400	≥1,000	≤4.0	≤0.5	Higher activity with caseinase to collagenase ratio ~2:1, designated to be enriched for Type II ( <i>col H</i> ) collagenase relative to Type I ( <i>col G</i> )
CLS-7	≥1,000	≥2,000	≤8.0	≤0.5	Contains collagenase and caseinase activities 4X higher than collagenase Types 1 and 2
CLSS-1	≥125	≥200	≤4.0	≤0.5	0.22μ Filtered CLS-1 in 50mg & 1gm Vials
CLSS-2	≥125	≥200	≥3.5	≥0.1	0.22μ Filtered CLS-2 in 50mg & 1gm Vials
CLSS-3	≥100	≥50	≤3.0	≤0.3	0.22μ Filtered CLS-3 in 50mg Vials
CLSS-4	≥160	≥100	≤3.0	≤0.1	0.22μ Filtered CLS-4 in 50mg & 1gm Vials
CLSS-5	≥450	≥450	≤4.0	≤0.3	Higher collagenase and caseinase activities
CLSH	≥125	≥200	≤4.0	≤0.5	0.22μ Filtered, ≥22,500U CLS-1 & 30U ESL component of HIS kit
<b>Animal Free</b>					
CLSAFP	≥1,500	≤50	≤2.0	≤0.25	Chromatographically purified, Low Protease/Collagen Studies, Tissue Digestion combined with other proteases
CLSAFA	≥150	≥150	≤8.0	≥0.1	Balanced Activities/AF Stem Cell & Tissue Bioprocessing
CLSAFB	≥300	≥300	≤5.0	≤0.5	Higher Activities/AF Stem Cell & Tissue Bioprocessing
CLSAFC	≥200	≥150	≤3.0	≤0.1	Lower Protease Activities/AF Stem Cell & Tissue Bioprocessing
CLSAFD	≥600	≥600	≤5.0	≤0.5	High Activity CLS/CAS AF Stem Cell & Bioprocessing
CLSAFAS	≥150	≥150	≤8.0	≥0.1	0.22μ Filtered AF CLSAFA in 50mg vials
CLSAFBS	≥300	≥300	≤5.0	≤0.5	0.22μ Filtered AF CLSAFB in 50mg vials
CLSAFCS	≥200	≥150	≤3.0	≤0.1	0.22μ Filtered AF CLSAFC in 50mg vials
<b>STEMxyme® Animal Free Blends of Collagenase and Neutral Protease</b>					
STZ1	≥250	≥1,000	≤5.0	≤0.5	0.22μ Filtered CLSAFB & NPRO/AF Stem Cell & Tissue Bioprocessing
STZ2	≥250	≥2,000	≤5.0	≤0.5	0.22μ Filtered CLSAFB & NPRO/AF Stem Cell & Tissue Bioprocessing
<b>Chromatographically Purified</b>					
CLSPA	≥500	≤50	≤2.0	≤0.25	Chromatographically purified, Low Protease/Collagen Studies, Tissue Digestion combined with other proteases
CLSPANK	≥500	≤50	≤2.0	≤0.25	0.22μ Filtered, ≥1,500U CLSPA component of NCIS kit



\* Correlations between type and effectiveness with different tissues have been good, but not perfect, and may be dependent partly on parameters of use and objectives as well as lot-to-lot variations. For more information see the Collagenase Sampling Program information.