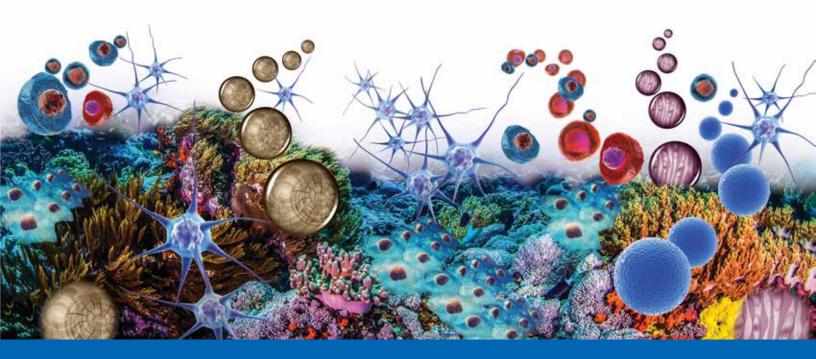
# **Stem Cell Research Products**



**COLLAGENASE** 

**DNASE 1** 

**PAPAIN** 

STEMxyme® COLLAGENASE/NEUTRAL PROTEASE BLENDS

PAPAIN (NEURAL) DISSOCIATION SYSTEM

**NEUTRAL PROTEASE (DISPASE®)** 

CELASE® GMP



# **Working Together**

# **Opening the Potential for Stem Cell Research Innovation**

# **Sharing Our Enzyme Expertise**

Is a top priority at Worthington, and we have done so for over 70 years. Authoring technical manuals and guides both in print and digital formats. As well, we are cited in thousands of respected scientific journals across the globe.

# To Support Your Research, We Provide Online Resources To Include:

- Advanced tissue search feature in the tissue dissociation guide section of our website
- Comprehensive citations listings at: Bioz.com by search for Worthington products to obtain article snippets with technique filtering capabilities
- Collagenase Sampling Program to pre-test a particular lot of enzyme you are planning to use in your experiment. This free service allows you to pre-sample several different lots of collagenase at a time and select the best of the group for the application.

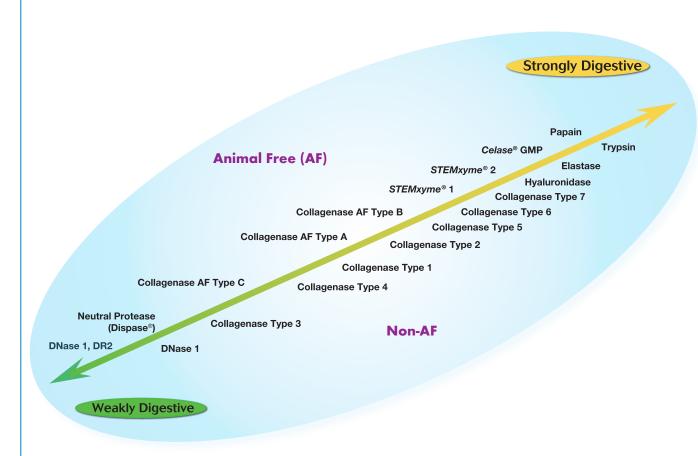
We invite you to work with us hand-in-hand to enhance our stem cell research technical library by submitting protocols, citations and articles referencing Worthington enzymes that can be shared with your colleagues. For details on submissions, contact your local Worthington Account Manager or forward suggestions to: techservice@worthington-biochem.com.



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# **Worthington Primary Cell Isolation Enzyme Digestion Scale**



Tissue dissociation/primary cell isolation and cell harvesting are principal applications for enzymes in tissue culture, stem cell research and cell biology studies. The goal of a cell isolation procedure is to maximize the yield of functionally viable, dissociated cells. There are many parameters which may affect the outcome. The choice of enzyme is an important parameter. Worthington's Tissue Dissociation Guide summarizes our knowledge of how these enzymes accomplish the "routine" operations of tissue dissociation and primary cell harvesting. This technical guide describes standard lab procedures; offers a logical experimental approach for establishing a cell isolation protocol; and lists many tissue specific references to aid development of an effective method. For more information, go to: **TissueDissociation.com** 

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# Celase® GMP Collagenase Blend

Source: Cl. histolyticum Collagenase/Bacillus Neutral Protease

I.U.B.: 3.4.24.3/3.4.24.28 CAS Number: 42613-33-2

**Celase® GMP** is a proprietary, blended proteolytic enzyme designed for efficient, gentle and reproducible *in vitro* dissociation of nucleated cells from adipose tissue.

#### **Convenience In Your Lab**

- A single, sterile, ready-to-use vial containing both collagenase and a neutral protease can digest up to 280 gm of adipose tissue
- · Best-in-class shelf life of up to 72 months

#### **Clarity In Your Approach**

- Research protocols are available from Cytori for dissociating canine, equine, human, ovine, porcine, rabbit and rodent adipose tissue
- Technical dossier is available from Cytori to ease the transition from research to clinical applications

#### **Confidence In Your Result**

- Included in IDE applications approved by U.S. FDA for alopecia, chronic heart failure, hamstring injuries, osteoarthritis of the knee, and hand manifestations of scleroderma
- Produced using avian and mammalian tissue-free raw materials, aseptic processes and sterile filtration under GMP guidelines to assure the lowest levels of impurities

**Total Protein** 34.4 - 51.6 mg/vial **Endotoxin** < 50 EU / mg

**Stability** Lyophilized: 72 months at -25 to -15°C

Reconstituted: 6 months at -25 to -15°C and up to 2 freeze-thaw cycles

**Appearance** White lyophilizate

Celase® CLAS

A single, sterile, ready-to-use vial containing both collagenase and a neutral protease which can digest up to 280 gm of adipose tissue. Stable up to 72 months at -20°C. REQUIRES SPECIAL SHIPPING AND PACKAGING: DRY ICE.

Digests ≥ 280 gm 1235-01 1 vial, 35 mg of adipose tissue



Expanding our commitment to convenience, clarity and confidence with the Celase® enzyme blend for pre-clinical applications.

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Catalog
Name Activity Number Package Code

# **Cell Isolation Optimizing System**

A complete method development kit containing an assortment of enzymes most frequently used in enzymatic tissue dissociation and cell isolation procedures. Includes instructions, references, and strategies for the handling, use and optimization of enzymatic cell isolation methods for maximum yield of viable cells. Contains all enzymes commonly referenced in tissue dissociation and cell isolation procedures. Also contains the **Cell Isolation Guide** which describes the tissue types commonly used, the mode of action of the various enzymes, tissue culture techniques, and protocol optimization guidelines (with cell- and tissue-specific references for getting started in enzymatic cell isolation).

Tissue dissociation and cell harvesting are two principal applications for enzymes in tissue culture research and cell biology studies. Despite the widespread use of enzymes for these applications over the years, their mechanisms of action in dissociation and harvesting are not well understood. As a result, the choice of one technique over another is often arbitrary and based more on past experience than on an understanding of why the method works and what modifications could lead to even better results.

Investigators searching the scientific literature for information on the ideal enzymes and optimal conditions for tissue dissociation are often confronted with conflicting data. Much of the variation stems from the complex and dynamic nature of the extracellular matrix and from the historical use of relatively crude, undefined enzyme preparations for cell isolation applications. The extracellular matrix is composed of a wide variety of proteins, glycoproteins, lipids and glycolipids, all of which can differ in abundance from species to species, tissue to tissue and with developmental stage. The Worthington Cell Isolation Optimizing System provides an assortment of the widely used enzymes in purified form for establishing an optimized cell isolation procedure on a cost-efficient basis.

#### **Kit Contents:**

- Collagenase Type 1, CLS-1, 500 mgdw
- Collagenase Type 2, CLS-2, 500 mgdw
- Collagenase Type 3, CLS-3, 500 mgdw
- Collagenase Type 4, CLS-4, 500 mgdw
- Trypsin, TRL, 500 mgdw
- Neutral Protease (Dispase®), NPRO, 10 mgdw

- Hyaluronidase, HSE, 50,000 un
- Elastase, ESL, 100 mgP
- Papain, PAPL, 100 mgP
- Deoxyribonuclease I, DP, 25 mgdw
- Trypsin Inhibitor, SIC, 100 mgdw

**Cell Isolation Optimizing System** 

A complete method development kit containing N/A an assortment of enzymes most frequently used in tissue dissociation and cell isolation procedures. Includes instructions, references, and strategies or the handling, use and optimization of enzymatic cell isolation methods to achieve maximum yield of viable cells. Kit includes 500 mg of each of four types of collagenase, 500 mg trypsin, 50 ku hyaluronidase, 100 mg elastase, 100 mg papain, 25 mg DNase I, 10 mg neutral protease (Dispase®) and 100 mg trypsin inhibitor. Store at 2-8°C.

LK003200 1 bx

CIT

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#### **Chymotrypsin**

Source: Bovine Pancreas

#### I.U.B.: 3.4.21.1 CAS Number: 9004-07-3

Chymotrypsin preferentially catalyzes the hydrolysis of peptide bonds involving L-isomers of tyrosine, phenylalanine and tryptophan. It also readily acts upon amides and esters of susceptible amino acids. Chymotrypsin catalyzes the hydrolysis of bonds of leucyl, methionyl, asparaginyl and glutamyl residues.

**Stability/Storage::** The enzyme is stable for days in solution at pH 3.0 and for years as a dry powder at 2-8°C. Protect from moisture.

**Unit Definition**: One Unit hydrolyzes one micromole of benzoyl-L-tyrosine ethyl ester per minute at 25°C, pH 7.8 in the presence of calcium. An activity of 45 Units per mg using the above definition, is the equivalent of 10,000 optical density or 1330 N.F. units per mg using ATEE as a substrate.

#### 1 BTEE unit = 29.5 USP/NF units.

Chymotrypsin, Alpha, TLCK Treated, Sequencing Grade Three times crystallized and treated with 1-chloro-3-tosylamido-7-amino-2-heptanone (TLCK) to inhibit trypsin activity (Shaw, et al., Biochemistry, 4, 2219, 1965). Dialyzed against 1 mM HCl to remove autolysis products and low molecular weight contaminants. Supplied lyophilized in 25 ug and 100 ug high-recovery vials. Store at 2-8°C.	≥ 45 Units per mg protein	LS02130 LS02132	4 x 25 ug 4 x 100 ug	CDSEQ
Chymotrypsin, Alpha, TLCK Treated Three times crystallized and treated with 1-chloro-3-tosylamido-7-amino-2-heptanone (TLCK) to inhibit trypsin activity (Shaw, et al., Biochemistry, 4, 2219 1965). Dialyzed against 1 mM HCl to remove autolysis products and low molecular weight contaminants. Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	≥ 45 Units per mg protein	LS001430 LS001432 LS001434 LS001438	25 mg 100 mg 1 gm Bulk	CDTLCK
<b>Chymotrypsin, Alpha, Purified</b> Chromatographically prepared by the procedure of Yapel <i>et al., J. Amer. Chem. Soc., 88,</i> 2573 (1966). A lyophilized powder. Store at 2-8°C.	≥ 45 Units per mg protein	LS001475 LS001479 LS001477	100 mg 1 gm Bulk	CDS
Chymotrypsin, Alpha, 3X Three times crystallized alpha chymotrypsin, which is an activation product of a three times crystallized zymogen. Dialyzed against 1 mM HCl and lyophilized. Store at 2-8°C.	≥ 45 Units per mg protein	LS001448 LS001450 LS001451 LS001453	250 mg 1 gm 10 gm Bulk	CDI
<b>Chymotrypsin, Alpha, Crystallized</b> Crystallized as zymogen and activated. Dialyzed against 1 mM HCl and lyophilized. Store at 2-8°C.	≥ 35 Units per mg protein	LS001333 LS001334 LS001332	1 gm 10 gm Bulk	CDAG

# Clostripain (Endoproteinase-Arg-C)

Source: Clostridium histolyticum

Name

I.U.B.: 3.4.22.8 CAS Number: 9028-00-6

Clostripain (Endoproteinase-Arg-C) is a two chain cysteine proteinase associated with collagenase and isolated from *Clostridium histolyticum*. It is highly specific for the carboxyl peptide bond of arginine. Clostripain is activated by dithiothreitol, cysteine, or other sulfhydryl containing reagents. The presence of calcium ions is essential. The enzyme is inhibited by oxidizing agents, divalent cations such as Co<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, and heavy metal ions. Citrate, borate, and Tris anions are less inhibitory.

**Unit Definition**: One Unit hydrolyzes one micromole of N-benzoyl-L-arginine ethyl ester per minute at 25°C, pH 7.6, in the presence of dithiothreitol.

Clostripain (Endoproteinase-Arg-C)				CPSEQ
Sequencing Grade				
Chromatographically purified. A dialyzed,	≥ 50 Units per	LS02135	10 ug	
pre-activated, lyophilized powder. Supplied in 10 µg high recovery vials. Store at 2-8°C.	mg protein	LS02139	Bulk	
Clostripain (Endoproteinase-Arg-C)				СР
Chromatographically purified. A dialyzed,	≥ 50 Units per	LS001641	1 mg	
pre-activated, lyophilized powder.	mg dry weight	LS001643	5 x 1 mg	
Store at 2-8°C.		LS001646	10 mg	
		LS001647	Bulk	



Our mission is to provide superior tools from discovery research through larger scale bioprocessing applications.

Worthing	ton Colla	igenase	Produ	cts, Sp	pecifications and Applications Table
Dundant Onda	Collagenase	Caseinase	Clostripain	Tryptic	Commonts (Applications)
Product Code	CDU/mgdw	u/mgdw	u/mgdw	u/mgdw	Comments/Applications*
Partially Purified					
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	≤3.0	≤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 (col H) collagenase relative to Type I (col G)
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	≤3.0	≤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
Animal Free					
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
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
STEMxyme® A	nimal Free Ble	ends of Colla	agenase and	d Neutral P	rotease
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
Chromatographic	ally Purified				
CLSPA	≥500	≤50	≤2.0	≤0.25	Low Protease/Collagen Studies, Tissue Digestion combined with other proteases
1					

<sup>\*</sup>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.

≤0.25

0.22µ Filtered, ≥1,500U CLSPA component of NCIS kit

≤50

≥500

≤2.0

**CLSPANK** 

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Name Activity Number Package Code

# Collagenase

Source: Clostridium histolyticum

I.U.B.: 3.4.24.3 CAS Number: 9001-12-1

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 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<sup>2+</sup> 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.
- CLSPA Chromatographically purified collagenase, contains minimal secondary proteolytic activities along with high
  collagenase activity. Animal Free Types AFA, AFB and AFC collagenase 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.

#### **Animal Free:**

- 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.

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.

The collagenase assay is a modification of Mandl wherein collagenase is incubated for five hours with native collagen and the extent of collagen breakdown is determined using the Moore and Stein, *J. Biol. Chem., 176, 367 (1948)* colorimetric ninhydrin method. Amino acids released are expressed as micromoles leucine per milligram collagenase.

**Uses:** Partially purified collagenases are widely used in enzymatic primary cell isolation and tissue dissociation procedures. Most researchers employ either partially purified collagenase preparations such as Types 1–7 or chromatographically purified collagenase (Code: **CLSPA**); the latter is usually combined with secondary enzymes such as elastase, hyaluronidase, etc. For best results, the precise mixture of proteolytic activities must be tailored to the tissue to be dissociated. 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 at the beginning of this catalog. Worthington also publishes a Tissue Dissociation Guide, which provides additional information regarding the enzymes used for these applications and specific references for numerous cell and tissue types. A complete copy is available on our website, Worthington-Biochem.com or may be requested through Customer Service at 800.445.9603/732.942.1660.

#### **Collagenase Lot Selection Tool Available Online**

Worthington's Collagenase Lot Selection Tool is 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, clostripan, and tryptic activities or specify previous lot numbers. Each value can be weighted based on 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 *techservice @Worthington-Biochem.com* to assist with collagenase or any other products.

# **Collagenase (Continued)**

Unit Definition: One unit releases one micromole of L-leucine equivalents from collagen in 5 hours at 37°C, pH 7.5.

Collagenase, Purified Chromatographically purified. ≤ 50 caseinase units per milligram. Supplied as a lyophilized powder. Store at 2-8°C.	≥ 500 units per mg dry weight	LS005275 LS005273 LS005277	4 ku 10 ku Bulk	CLSPA
Collagenase Vial, NCIS 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 fo cell isolation and culture applications. Store at 2-8°C.		LK003240 LK003245	1 vi 5 vi	CLSPANK
Collagenase, Type 1 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 powstore at 2-8°C.	≥ 125 units per mg dry weight der.	LS004194 LS004196 LS004197 LS004200	100 mg 1 gm 5 gm Bulk	CLS-1
Collagenase, Type 2 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.	≥ 125 units per mg dry weight	LS004174 LS004176 LS004177 LS004179	100 mg 1 gm 5 gm Bulk	CLS-2
Collagenase, Type 3 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.	≥ 100 units per mg dry weight	LS004180 LS004182 LS004183 LS004185	100 mg 1 gm 5 gm Bulk	CLS-3
Collagenase, Type 4 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.	≥ 160 units per mg dry weight	LS004186 LS004188 LS004189 LS004191	100 mg 1 gm 5 gm Bulk	CLS-4
Collagenase, Type 5 Prepared to contain higher collagenase and caseinase activities. A dialyzed, lyophilized powder. Store at 2-8°C.	≥ 450 units per mg dry weight	LS005280 LS005282 LS005283 LS005284	100 mg 1 gm 5 gm Bulk	CLS-5

Name	Activity	Catalog Number	Package	Code
Collagenase (Continued)				
Collagenase, Type 6 Prepared to contain 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). A dialyized, lyophilized powder.  Store at 2-8°C.	≥ 400 units per mg dry weight	LS005318 LS005319 LS005321 LS005323	100 mg 500 mg 2.5 gm Bulk	CLS-6
Collagenase, Type 7 Prepared to contain collagenase and caseinase activities four-fold higher than collagenase Type 1/2. A dialyized, lyophilized powder. Store at 2-8°C.	≥ 1,000 units per mg dry weight	LS005332 LS005333 LS005335 LS005337	100 mg 500 mg 2.5 gm Bulk	CLS-7
Collagenase, Type 1, 0.22µ Filtered 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	LS004214 LS004216 LS004217	50 mg 5 x 50 mg 1 gm	CLSS-1
Collagenase, Type 2, 0.22μ Filtered 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	LS004202 LS004204 LS004205	50 mg 5 x 50 mg 1 gm	CLSS-2
Collagenase, Type 3, 0.22μ Filtered 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	LS004206 LS004208	50 mg 5 x 50 mg	CLSS-3
Collagenase, Type 4, 0.22μ Filtered 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	LS004210 LS004212 LS004209	50 mg 5 x 50 mg 1 gm	CLSS-4
Collagenase, Type 5, 0.22μ Filtered 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	LS005286 LS005287 LS005288	50 mg 5 x 50 mg 1 gm	CLSS-5
Collagenase/Elastase Vial, HIS Kit 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 unreconstituted vials at 2–8°C.	≥ 20,000 units per vial	LK002066 LK002067	1 vi 5 vi	CLSH

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# STEMxyme® Collagenase/Neutral Protease Blends, Animal Free

#### STEMxyme®1, Collagenase/Neutral Protease (Dispase®), 0.22 Filtered Animal Free

A specialized combination of Animal Free *Clostridium* histolyticum collagenase and Animal Free Bacillus polymyxa 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

 $\geq$  250 collagenase units LS004106 50 mg per mg dry weight LS004107 5 x 50 mg

≥ 1,000 caseinase units per mg dry weight

STZ1

STZ2



#### STEMxyme<sup>®</sup>2, Collagenase/Neutral Protease (Dispase<sup>®</sup>), 0.22 Filtered Animal Free

A specialized combination of Animal Free *Clostridium*histolyticum collagenase and Animal Free Bacillus polymyxa
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.

 $\geq$  250 collagenase units LS004112 50 mg per mg dry weight LS004113 5 x 50 mg

≥ 2,000 caseinase units per mg dry weight



Animal free enzymes, exceeding expectations and meeting industry standards – quality assurance lot-to-lot.

**Activity** 

# **Collagenases, Animal Free**

				<u> </u>
CLSAFA				Collagenase, Animal Free, Type A
ANIMA.	100 mg 1 gm 5 gm Bulk	LS004152 LS004154 LS004156 LS004158	≥ 150 units per mg dry weight	Collagenase derived from cultures grown in animal free medium. Suitable for applications needing to avoid introduction of animal derived pathogens into bioprocessing procedures. Store at 2-8°C.
CLSAFAS				Collagenase, Animal Free
AREE.	50 mg 5 x 50 mg	LS004118 LS004119	≥ 150 units per mg dry weight	<b>Type A, 0.22 Filtered</b> Collagenase, Animal Free which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.
CLSAFB				Collagenase, Animal Free,
ANIMA	100 mg 1 gm 5 gm Bulk	LS004145 LS004147 LS004148 LS004150	≥ 300 units per mg dry weight ng	<b>Type B</b> Prepared from cultures grown in medium completely devoid of animal based components and designed for bioprocessir applications where introduction of animal derived pathogens must be prevented. Store at 2-8°C.
CLSAFBS				Collagenase, Animal Free,
NIMAY.	50 mg 5 x 50 mg	LS004124 LS004125	≥ 300 units per mg dry weight	<b>Type B, 0.22 Filtered</b> Collagenase, Animal Free which is filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C.
CLSAFC				Collagenase, Animal Free,
ANIMAC.	100 mg 1 gm 5 gm Bulk	LS004138 LS004140 LS004141 LS004143	≥ 200 units per mg dry weight	Type C 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.  Store at 2-8°C.
CLSAFCS				Collagenase, Animal Free,
NIMAX.	50 mg 5 x 50 mg	LS004130 LS004131	≥ 200 units per mg dry weight	Type C, 0.22 Filtered Collagenase Animal Free which is filtered through a 0.22 micron membrane and lyophilized in vials

Store at 2-8°C.

# **Deoxyribonuclease I**

Source: Bovine Pancreas

#### I.U.B.: 3.1.21.1 CAS Number: 9003-98-9

Bovine pancreatic deoxyribonuclease is an endonuclease that splits phosphodiester linkages, preferentially adjacent to a pyrimidine nucleotide, yielding polynucleotides with free hydroxyl group at the 3' position and a phosphate group at the 5' position. The average chain length of a limit digest is a tetranucleotide.

**Uses:** Worthington offers DNase at different levels of purity for different applications. Product Codes: DPRF and DPRFS are both especially designed for Molecular Biology applications and contain the lowest levels of RNase and protease activity. They are both suitable for use in techniques requiring digestion of DNA in the recovery of intact RNA or where the integrity of structural proteins or enzymes must be maintained. Applications have included nick translation, DNA mapping, isolation of nuclear RNA and protein, RNA polymerase synthesis of RNA probes and RT-PCR. DNase is also used in tissue culture work to digest DNA from damaged cells thereby reducing viscosity, and removing membrane bound DNA fragments. Worthington Codes: DP and DCLS are suitable for these applications.

**Stability/Storage:** When properly stored, all grades of Worthington deoxyribonuclease are stable for 2-3 years. Product code DPRFS may be stored at -20°C. For long term storage in solution, Product Codes D and DPFF may be dissolved in 5 mM acetate, 1 mM calcium, pH 4.5 and stored in single use aliquots at -20°C or -70°C for up to one year. Only freeze and thaw once; thawed aliquots are stable refrigerated at least several weeks. Addition of 50% glycerol will maintain a liquid state at -20°C without affecting stability. Material in 50% glycerol can be removed and returned to -20°C repeatedly. DPRF is unusually stable due to the absence of protease. For long term storage of DPRF after reconstitution, use water or any buffer pH 4.0 to 9.0 except phosphate; add 50% glycerol for storage as liquid at -20°C; avoid calcium chelators. Aliquot in single use containers; only freeze and thaw once; thawed aliquots are stable refrigerated at least several weeks.

**Unit Definition**: 1 unit causes an increase in absorbance at 260 nm of 0.001 per minute per ml at 25°C when acting upon highly polymerized DNA at pH 5.0. **Note**: Kunitz units as reported by other suppliers can be 2 to 4 times higher than Kunitz units as measured at Worthington. As measured at Worthington, one Kunitz unit digests 1  $\mu$ g of calf thymus DNA in 10 minutes at 37°C in 50 mM Tris, 1 mM Mg<sup>2+</sup>, 1 mM Ca<sup>2+</sup>, pH 7.8. Correlation of digestion units with Kunitz units is different for other DNA and buffer systems.

**Technical Note**: Product Code DPRF: Each vial contains approximately 2 mg glycine and 2 μmoles calcium per 10,000 units of DNase I. Dissolving the entire vial in 5 ml provides the equivalent of a 1 mg/ml solution.



From research and development to manufacturing, continuous quality improvement is everyone's job.

Name	Activity	Catalog Number	Package	Code
Deoxyribonuclease I (Continued)				
Deoxyribonuclease I, Ribonuclease & P Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Supplied as a solution at approximately 2 Kunitz units per microliter approximately 1 mg/ml containing 50% glycerol and 1 mM calcium chloride. Store at 2-8°C or -20°C.	rotease Free, So ≥ 2,000 Kunitz units per ml	lution LS006342 LS006344 LS006348	100 un 500 un Bulk	DPRFS
Deoxyribonuclease I, Ribonuclease & P Molecular Biology Grade. Chromatographically purified to remove RNase and protease. Lyophilized in vials. Each 10,000 unit vial contains 2 mg glycine, 2 μmoles calcium, and ≥ 10,000 units of DNase I. Each 2,500 unit vial contains 0.5 mg glycine, 0.5 μmoles calcium and ≥ 2,500 units of DNase I. Dissolving the entire 10,000 unit vial in 5 ml, or the entire 2,500 unit vial in 1.25 ml, provides the equivalent of a 1 mg/ml solution. (ku = 1000 Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 2,000 Kunitz units per mg dry weight	LS006331 LS006333 LS006343 LS006334	2500 un 10 ku 50 ku Bulk	DPRF
Deoxyribonuclease I Chromatographically purified. A lyophilized powder containing glycine as a stabilizer. Protease Free. Contains ≤ 0.0005% RNase. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 2,000 Kunitz units per mg dry weight	LS006330 LS006328 LS006332	25 ku 125 ku Bulk	DPFF
<b>Deoxyribonuclease I</b> Chromatographically purified. A lyophilized powder with glycine as a stabilizer. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 2,000 Kunitz units per mg dry weight	LS002004 LS002006 LS002007 LS002009	5 mg 20 mg 100 mg Bulk	D
<b>Deoxyribonuclease I, Filtered</b> Filtered through a 0.22 micron membrane and lyophilized in vials. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 2,000 Kunitz units per mg dry weight	LS002058 LS002060	11 mg 25 mg	DCLS
Deoxyribonuclease I, Standard Vial Lyophilized in vials for assay standardization. Labeled to show established activity. Not suitable for assays at neutral pH. Store at 2-8°C.	~2,000 Kunitz units per vial	LS002173 LS002172	2 ku 5x2 ku	DSV

Name	Activity	Catalog Number	Package	Code
Deoxyribonuclease I (Continued)				
PDS Kit, DNase Vial A component of the Papain Dissociation System. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5 ml of EBSS or equivalent yields a solution of 2000 units/ml of deoxyribonuclease (1 mg/ml). Store at 2-8°C.	≥ 1,000 units per vial	LK003170 LK003172	1 vi 5 vi	D2
<b>Deoxyribonuclease I</b> Partially purified. A lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 2,000 Kunitz units per mg dry weight	LS002138 LS002139 LS002140 LS002141	25 mg 100 mg 1 gm Bulk	DP
<b>Deoxyribonuclease I</b> Partially purified. A lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 1,250 Kunitz units per mg dry weight	LS002145 LS002147 LS002149	100 mg 1 gm Bulk	DPB



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#### **Elastase**

Source: Porcine Pancreas

#### I.U.B.: 3.4.21.36 CAS Number: 39445-21-1

Porcine pancreatic elastase has a molecular weight of 25.9 kDa, and a pH optimum of 8.5. While elastase will hydrolyze a wide variety of protein substrates, it is unique among proteases in its ability to hydrolyze native elastin, a substrate not attacked by trypsin, chymotrypsin or pepsin. Soybean trypsin inhibitor and kallikrein inhibitor suppress proteolytic but not elastolytic activity. Elastase is assayed using a method adapted from that of Feinstein *et al.*, *Biochem. Biophys. Res. Comm.*, *50*, 1020 (1973) and using the more soluble substrate of Bieth *et al.*, *Biochem. Med.*, *11*, 350 (1974).

**Stability/Storage:** Elastase is unstable at pH  $\leq$  3.5. When stored as a dry powder the enzyme is stable for 6-12 months at 2-8°C. Elastase product codes: ES and ESL have poor solubility at neutral pH and at concentrations greater than 0.25%. It is helpful to make primary solutions in KCl or alkaline buffers before diluting into the reaction mixtures or media, compensating for ionic strength or pH changes. Stable at pH 4.0-10.4.

**Technical Notes**: 1 SucAla<sub>3</sub>NA unit is approximately equivalent to 6 elastin digestion units. Aqueous liquid suspensions should be aseptically handled to avoid bacterial contamination. Due to the viscous nature of the aqueous suspension (Code: ES) the vial should be rinsed to recover contents.

**Unit Definition**: One Unit cleaves one micromole of N-succinyl-L-alanyl-L-alanyl-L-alanine-p-nitroanilide per minute at 25°C, pH 8.0.

Elastase, Purified Chromatographically purified. A lyophilized powder. Store at 2-8°C. REQUIRES SPECIAL SHIPPING: ICE PAC	≥ 8 Units per mg protein K	LS006363 LS006365 LS006367	5 mg 20 mg Bulk	ESFF
Elastase, Lyophilized Two times crystallized, (Code: ESL), supplied as a dialyzed, lyophilized powder. The enzyme should be 0.22 micron filtered after reconstitution and prior to use. Suitable for the isolation of Type II lung cells. Store at 2-8°C. Does not require special shippping.	≥ 3 Units per mg protein	LS002290 LS002292 LS002294 LS002298	25 mg 100 mg 1 gm Bulk	ESL
Elastase, Suspension Two times crystallized. Supplied as an aqueous suspension. This preparation must be diluted to dissolve the enzyme. The diluted enzyme should be 0.22 micron filtered before use. Suitable for the isolation of Type II lung cells. Store at 2-8°C. DO NOT FREEZE. REQUIRES SPECIAL SHIPPING: ICE PAC	≥ 3 Units per mg protein	LS002274 LS002279 LS002280 LS002276	25 mg 100 mg 1 gm Bulk	ES

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Catalog
Name Activity Number Package Code

# **Hepatocyte Isolation System**

Most traditional methods published for isolating hepatocytes use crude and partially purified enzyme preparations including various types of collagenase and other proteases. More recently the use of better characterized preparations of collagenase such as Worthington Types 1 - 7 (CLS-1-7) have provided better results. All partially purified collagenase preparations can contain lot-variable contaminating proteases, esterases and other enzymes requiring researchers to pre-screen several lots of enzyme and/or continually modify isolation parameters and protocols.

The Worthington Hepatocyte Isolation System has been developed to provide researchers with a reliable, convenient, and consistent hepatocyte cell isolation system. By using the pre-optimized combination of enzymes contained in this kit, it is possible to minimize the lot-to-lot variation and improve the quality of the isolated hepatocytes. In addition, Worthington use-tests each lot by isolating hepatocytes from adult rat to assure performance, reliability, and consistent yield of viable cells. The method is based on that described by Berry *et al.*, and modified by Seglen, *Methods in Cell Biology*, *13* (Prescott, D. ed.), Academic Press, 29 (1976), and further optimized in conjunction with several researchers.

**Stability/Storage:** The reagents are stable at ambient temperatures for the periods of time expected in normal shipping procedures, but the package should be refrigerated upon arrival. Contents may be stored at 2-8°C for 4-6 months before use. Store at 2-8°C.

**Package Contents:** The package contains sufficient materials for five separate adult rat liver perfusions. For larger or smaller tissue applications, prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

- Vial #1: 10X CMF-HBSS Concentrate, 1 bottle, 500 ml Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF-HBSS). The solution is used for washing and perfusing the liver prior to the addition of the dissociating enzyme solution.
- Vial #2: Collagenase/Elastase Enzyme Vial, 5 vials Containing collagenase (Code: CLS-1) and elastase (Code: ESL) ≥ 20,000 u/vial and ≥ 30 u/vial respectively. Before use, reconstitute with the L-15/MOPS solution and swirl gently to dissolve contents. Store unreconstituted vials at 2-8°C.
- Vial #3: 1,000 units DNase I each, 5 vials

Worthington DNase I (Code: D), filtered through 0.22 µm pore size membrane, and lyophilized. Before use, reconstitute with L-15/MOPS solution and swirl gently to dissolve contents. Store unreconstituted vials at 2-8°C.

- Vial #4: 0.15 M MOPS, pH 7.5, 1 bottle, 75 ml 0.15 M MOPS, pH 7.5 buffer concentrate, used to buffer the reconstituted Leibovitz L-15 media.
- **Vial #5:** 7.5% Sodium Bicarbonate (NaHCO<sub>3</sub>), 1 bottle, 100 ml 7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS.
- Pouch, containing Leibovitz L-15 Media Powder, 1 x 1L

Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing approximately 800 ml of cell culture grade water. Rinse pouch 2 - 3 times with an additional 100 ml water. Bring total volume to 1000 ml and filter through a 0.22 micron membrane.

LK002060

1 bx

N/A

#### **Hepatocyte Isolation System**

HIS

The package contains sufficient materials for five separate adult rat liver perfusions including five single use CLSH enzyme vials, five single use DNase vials, 10X CMF-Hank's Balanced Salt Solution, L-15 Media Powder, 0.15 M MOPS buffer. 7.5% sodium bicarbonate solution and optimized protocol. Store at 2-8°C.

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# **Hepatocyte Isolation System (Continued)**

Collagenase/Elastase Vial, HIS Kit Worthington collagenase (Code: CLS-1) and elastase (Code: ESL), filtered through 0.22 µm pore size membrane, and lyophilize Before use, reconstitute with the L-15/MOPS solution and swirl gently to dissolve contents Store unreconstituted vials at 2–8°C.	6	LK002066 LK002067	1 vi 5 vi	CLSH
DNase Vial, HIS Kit A component of the Hepatocyte Isolation kit containing 1,000 units DNase I each, 5 vials Worthington DNase I (Code: D), filtered through 0.22 μ pore size membrane, and lyophilized. Before use, reconstitute with L-15/MOPS solution and swirl gently to dissolve contents.  Store unreconstituted vials at 2–8°C.	≥ 1,000 units per vial	LK003170 LK003172	1 vi 5 vi	D2

Hank's Balanced Salt Solution (HBSS-CMF) 10X Solution, HIS Kit LK002064 N/A

**HBSS10** 

1 ea

10X CMF-HBSS Concentrate, 1 bottle, 500 ml. Sterile calcium- and magnesiumfree Hank's Balanced Salt Solution (CMF-HBSS). The solution is used for washing and perfusing the liver prior to the addition of the dissociating enzyme solution. Store at 2-8°C.



We keep a close eye on citations both peer-reviewed articles and open access publications to assist you in choosing the best protocol and products for your research.

Name	Activity	Catalog Number	Package	Code
Hepatocyte Isolation System (Contin	ued)			
L-15 Media Powder, HIS Kit Leibovitz L-15 media powder, a component of the HIS kit. Reconstitute entire contents of pouch, QS to 1 liter with cell culture grade water, and 0.22 micron filter. Suitable for cell isolation and culture applications. Store at 2-8°C.	N/A	LK003250	1 ea	L15NK
<b>0.15 M, MOPS Buffer, HIS Kit</b> 0.15 M MOPS, pH 7.5, 0.22 μ filtered. Buffer concentrate used to buffer the constituted Leibovitz L-15 media in Hepatocyte Isolation System. Store at 2-8°C.	N/A	LK002070	1 ea	MOPS
<b>Sodium Bicarbonate, 7.5%, HIS Kit</b> 7.5% Sodium Bicarbonate (NaHCO <sub>3</sub> ), 1 bottle, 100 ml 7.5% sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS. Store at 2-8°C.	N/A	LK002069	1 ea	NAH

Name Activ	Catalog ity Number Package	Code
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# Hyaluronidase

Source: Bovine Testes

I.U.B.: 3.2.1.35 CAS Number: 37326-33-3

Testicular hyaluronidase is a glycoprotein containing 5% mannose and 2.7% glucosamine. Optimum pH range is 4.5-6.0. The enzyme catalyzes the hydrolysis of endo-N-acetylhexosaminic bonds of hyaluronic acid and chondroitin sulfate A and C (but not B), primarily to tetrasaccharide residues.

**Unit Definition**: One unit is based on the change in absorbency (turbidity) at 540nm of an internal standard assayed concurrently with each lot. Internal standard replaces USP/NF reference no longer available.

Hyaluronidase				HSE
A partially purified, dialyzed,	≥ 300 units	LS002594	50 ku	
lyophilized powder.	per mg	LS002592	300 ku	
Store at -20°C.	dry weight	LS002591	Bulk	
Hyaluronidase, Purified				HSEP
Chromatographically purified.	≥ 3,000 units	LS005477	5 ku	
A dialyzed, lyophilized powder.	per mg	LS005475	15 ku	
Store at -20°C.	dry weight	LS005474	30 ku	
		LS005479	Bulk	

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# **Neonatal Cardiomyocyte Isolation System**

The Worthington Neonatal Cardiomyocyte Isolation System has been developed to provide researchers with a reliable, convenient, and consistent neonatal rat cardiomyocyte cell isolation method. By using purified, rather than crude enzyme preparations, it has been possible to minimize the lot-to-lot variation. In addition, Worthington use-tests the kits by isolating cardiomyocytes from neonatal rat hearts to assure performance, reliability and consistent yield of viable cells. The kit has been formulated in collaboration with Dr. Ronal MacGregor. The method is based on that described by Toraason *et al.*, *Toxicol.* 56, 107 (1988) in which the minced tissue is incubated overnight with purified trypsin at 2-8°C. As pointed out by Toraason, this step reduces the hands-on time required to harvest cells compared to the time involved in sequential incubations in warm trypsin or collagenase. Purified collagenase rather than crude collagenase is used to maximize yield and viability.

#### **Contents of Kit**

The package contains sufficient materials for five separate tissue dissociations, each containing up to twelve hearts. For larger or smaller tissue samples prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

- **Vial 1**: 1 bottle, 500 ml: Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF HBSS), pH 7.4. The solution is used for reconstituting the contents of Vials #2 and #3 in addition to serving as the medium for the dissociation.
- **Vial 2**: 5 vials, 1000 µg each: Worthington Trypsin (Code: TRLS), chromatographically purified, dialyzed against 1 mM HCl, filtered through 0.22 micron pore size membrane, and lyophilized. Before use, reconstitute with 2 ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2-8°C.
- **Vial 3:** 5 vials, 2000 µg each: Worthington Soybean Trypsin Inhibitor (Code: SIC), a 0.22 micron pore size membrane-filtered, lyophilized powder. Before use, reconstitute with 1 ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2-8°C.
- **Vial 4**: 5 vials, 1500 units each: Worthington Purified Collagenase (Code: CLSPA), a 0.22 micron pore size membrane-filtered, lyophilized powder which has been chromatographically purified. It contains less than 50 caseinase units per milligram and is composed of two separable but very similar collagenases. Before use, reconstitute with 5 ml Leibovitz L-15 media (prepared as described below) and swirl gently to dissolve contents. Store at 2-8°C.
- **Pouch Containing Leibovitz L-15 Media Powder**: 1 x 1L, Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing 800 ml of cell culture grade water. Rinse pouch 2-3 times with additional 100 ml. Bring total volume to 1 liter and filter through a 0.22 micron pore size filter.

The kit also includes 5 Cell Strainers (Falcon), a card correlating phenol red color with pH for checking balanced salt solutions and culture media.

Neonatal Cardiomyocyte Isolation System				NCIS
Kit for performing five separate tissue	N/A	LK003300	1 ki	
dissociations, each containing up to twelve		LK003303	3 ki	
hearts. Contains single use vials of purified				
collagenase and trypsin, CMF-HBSS,				
Leibovitz L-15 media and Falcon cell strainers				
along with a detailed protocol. The kit is use-				
tested by Worthington to assure performance.				
Store at 2-8°C.				

Store at 2-8°C.				
Collagenase Vial, NCIS 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.	≥ 1500 units per vial	LK003240 LK003245	1 vi 5 vi	CLSPANK

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Name	Activity	Catalog Number	Package	Code
leonatal Cardiomyocyte Isolation Sy	stem (Conti	nued)		
Trypsin Vial, NCIS A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 2 ml of HBSS yields a solution of 500 μg/ml of trypsin, Code: TRLS Suitable for cell isolation and culture application Store at 2-8°C.		LK003220 LK003225	1 vi 5 vi	TRLSNK
Inhibitor Vial, NCIS A component of the NCIS kit. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 1 ml of HBSS or equivalent yields a solution of 2 mg/ml of trypsin inhibitor, Code: SIC. Suitable for cell isolation and culture applications. Store at 2-8°C.	1 mg inhibits at least 0.75 mg trypsin Code: TRL	LK003230 LK003235	1 vi 5 vi	SICNK
HBSS Solution Sterile calcium and magnesium free Hank's balanced salt solution (CMFHBSS), pH 7.4, as supplied in the NCIS kit; 1 x 500 ml. Store at 2-8°C.	N/A	LK003210	1 ea	HBSS
L-15 Media Powder Leibovitz L-15 media powder, a component of the NCIS kit. Reconstitute entire contents of pouch, QS to 1 liter with cell culture grade water, and 0.22 micron filter. Suitable for cell isolation and culture applications. Store at 2-8°C.	N/A	LK003250	1 ea	L15NK
Cell Strainers (Falcon) Cell strainers (Falcon), components of the NCIS kit. Suitable for removal of tissue debris in cell isolation applications.	N/A	LK003265	5 ea	CELSTRNK

Store at room temperature.

# Neutral Protease (Dispase®), Animal Free

Source: Bacillus polymyxa

I.U.B.: 3.4.24.28 CAS Number: 42613-33-2

A metallo, neutral protease, purified by methods developed at Worthington. Its mild proteolytic action makes the enzyme especially suitable for the preparation of primary and secondary (subcultivation) cell culture. This protease is also used as a secondary enzyme in cell isolation and tissue dissociation applications.

**Stability/Storage:** Stable at 2-8°C for 12 months. Store at 2-8°C. After reconstitution with water or buffer, aliquot and store at -20°C.

**Unit Definition**: One Unit releases Folin positive amino acids equivalent to 1 micromole tyrosine per minute from casein at 37°C, pH 7.5.

Neutral Protease (Dispase®), Purified				NPRO
Chromatographically purified.	≥ 4 Units per	LS02100	10 mg	ANIMA
A lyophilized powder.	mg dry weight	LS02104	50 mg	FREE
Store at 2-8°C.		LS02106	250 mg	FREE!
		LS02108	Bulk	
Neutral Protease, Partially Purified				NPRO2
Partially purified. A lyophilized powder.	≥ 0.1 Units per	LS02109	1 gm	ANIMA
Store at 2-8°C.	mg dry weight	LS02111	5 gm	FREE
		LS02112	Bulk	*AREE

Name	Activity	Catalog Number	Package	Code

#### **Ovalbumin**

Source: Egg White

**CAS Number: 9006-59-1** 

Ovalbumin is a glycoprotein with molecular weight of 45 kDa. The molecule consists of a polypeptide with up to two phosphate groups per mole and a side chain of mannose and glucosamine residues.

<b>LowEndo™ Ovalbumin, Purified</b> Ovalbumin, purified to remove endotoxin. A dialyzed, lyophilized powder. Store at 2-8°C.	≥95% Purity (SDS-PAGE) ≤1 Endotoxin unit per mg	LS003059 LS003061 LS003062 LS003064	10 mg 100 mg 500 mg Bulk	OAEF
<b>Ovalbumin, Purified</b> Highly purified. Major protein of egg white, with a molecular weight of 45 kDa. A dialyzed, lyophilized powder. Store at 2-8°C.	N/A	LS003056 LS003054 LS003052	100 mg 1 gm Bulk	OAC
<b>Ovalbumin</b> Major protein of egg white, with a molecular weight of 45 kDa. A lyophilized powder. Store at 2-8°C.	N/A	LS003049 LS003048 LS003050	1 gm 5 gm Bulk	OA

Catalog
Name Activity Number Package Code

# **Papain**

Source: Carica papaya Latex

Papain, Suspension

#### I.U.B.: 3.4.22.2 CAS Number: 9001-73-4

Papain is a sulfhydryl protease from *Carica papaya* Latex. It has a molecular weight of 23 kDa and an optimum pH range of 6.0-7.0. The action of papain on leucine methyl ester produces an insoluble polyleucine peptide. Papain breaks down the intercellular matrix of cartilage. Papain is activated by cysteine, sulfide, and sulfite. Stabilizing agents are EDTA, cysteine and dimercaptoethanol.

**Stability/Storage:** Stable for 6-12 months at 2-8°C. Do not freeze aqueous suspensions.

**Technical Notes**: Papain preparations should be incubated in the activation solution before use to ensure full activity. Applications include antibody fragmentation and primary/neural cell isolation.

**Unit Definition**: One Unit hydrolyzes one micromole of benzoyl-L-arginine ethyl ester per minute at 25°C, pH 6.2, after activation in a solution containing 1.1 mM EDTA, 0.067 mM mercaptoethanol and 5.5 mM cysteine-HCl for 30 minutes.

Supplied as a 2X crystalline suspension in 50 mM sodium acetate, pH 4.5. To ensure full activity, the enzyme should be incubated in a solution containing 1.1 mM EDTA, 0.067 mM mercaptoethanol and 5.5 mM cysteine-HCl for 30 minutes. It is recommended that the enzyme be 0.22 micron filtered after dissolution and prior to Store at 2-8°C.  REQUIRES SPECIAL SHIPPING: ICE PA		LS003124 LS003126 LS003127 LS003128	25 mg 100 mg 1 gm Bulk	1.01
Papain, Lyophilized Supplied as a lyophilized powder prepared from a 2X crystalline suspension, Code: PAP. To ensure full activity, the enzyme should be incubated in a solution containing 1.1 mM EDTA, 0.067 mM mercaptoethanol and 5.5 mM cysteine-HCl for 30 minutes. It is recommended that the enzyme be 0.22 micron filtered after dissolution and prior to use. Store at 2-8°C.	Activates ≥15 Units per mg protein	LS003118 LS003119 LS003120 LS003122	25 mg 100 mg 1 gm Bulk	PAPL
PDS Kit, Papain Vial A component of the Papain Dissociation System, for use in the tissue dissociation method of Huettner, J., and Baughman, R., J. Neuroscience, 6, 3044 (1986). Contains papain, L-cysteine, and EDTA. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of EBSS or equivalent yields a solution at 20 Units of papain per ml in 1 mM L-cysteine with 0.5 mM EDTA. Store at 2-8°C.	≥ 100 Units per vial	LK003176 LK003178	1 vi 5 vi	PAP2

**PAP** 

# **Papain (Neural) Dissociation System**

The Worthington Papain Dissociation System is a set of reagents intended for use in the neural cell isolation method of Huettner and Baughman, J. Neurosci., 6, 3044 (1986). The materials are designed for convenience and simplicity and are useful to the occasional user as well as the more experienced and frequent user. Each lot is use-tested for performance in rat spinal neural cell isolation and this kit provides freshly prepared enzyme solutions for each dissociation.

Stability/Storage: The reagents are stable at ambient temperatures for the periods of time expected in normal shipping procedures, but the package should be refrigerated upon arrival. Contents may be stored at 2-8°C for 4 months before use. Store at 2-8°C.

#### **Package Contents**

The package contains sufficient materials for dissociation of five separate tissue aliquots of up to 0.3-0.4 cm<sup>3</sup> each. For larger tissue samples prepare proportionately larger volumes of reagents at each step and combine them in the same ratio as described in the protocol.

- Vial 1: Sterile Earle's Balanced Salt Solution (EBSS) with calcium, magnesium, bicarbonate and phenol red, one vial per package, 100 ml. Aliguots of this vial are used to reconstitute other vials and to prepare dilute inhibitor solution. Refrigerate between uses and equilibrate with sterile O<sub>2</sub>:CO<sub>2</sub> before each use.
- Vial 2: Papain containing L-cysteine and EDTA, 5 x 100 Unit single-use vials per package. The material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of EBSS (Vial 1) yields a solution at 20 Units of papain per ml in 1 mM L-cysteine with 0.5 mM EDTA. Brief incubation at 37°C is needed to insure full solubility and activity.
- Vial 3: Deoxyribonuclease I (DNase), 5 x 1000 unit single use vials per package. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5 ml of EBSS (Vial 1) yields a solution at 2000 units of deoxyribonuclease per ml. Avoid vigorous mixing.
- Vial 4: Ovomucoid protease inhibitor with bovine serum albumin, one vial per package, 32 ml upon reconstitution. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 32 ml of EBSS (Vial 1) yields a solution at an effective concentration of 10 mg of ovomucoid inhibitor and 10 mg of albumin per ml. Aliquots of this vial are used for each dissociation. Refrigerate between uses and equilibrate with sterile O<sub>2</sub>:CO<sub>2</sub> before each use. Stable after reconstitution when stored at 2-8°C.

Also included is a card correlating color with pH for use as a guide in O<sub>2</sub>:CO<sub>2</sub> equilibration.

Papain Dissociation System Set of five single use vials of papain and five single use vials of DNase, 100 ml of Earle's balanced salt solution (EBSS), and an inhibitor vial for use in the tissue dissociation method of Huettner and Baughman, <i>J. Neuroscience</i> , <i>6</i> , 3044 (1986). Use-tested by Worthington using new-born rat pup spinal cord. The package contains sufficient materials for dissociation of five separate tissue aliquots of up to 0.3-0.4 cm³ each. Store at 2-8°C.	N/A	LK003150 LK003153	1 bx 3 bx	PDS
Papain Dissociation System, Without EBS	SS			PDS2

Complete kit as described for product Code: N/A LK003160 1 bx PDS, but without the Earle's Balanced Salt LK003163 3 bx Solution (EBSS). Store at 2-8°C.

Name	Activity	Catalog Number	Package	Code
Papain (Neural) Dissociation System	(Continued)			
PDS Kit, Papain Vial A component of the Papain Dissociation System, for use in the tissue dissociation method of Huettner and Baughman, J. Neuroscience, 6, 3044 (1986). Contains papain, L-cysteine, and EDTA. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 5 ml of EBSS or equivalent yields a solution at 20 Units of papain per ml in 1 mM L-cysteine with 0.5 mM EDTA. Store at 2-8°C.	≥ 100 Units per vial	LK003176 LK003178	1 vi 5 vi	PAP2
PDS Kit, DNase Vial A component of the Papain Dissociation System. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5 ml of EBSS or equivalent yields a solution of 2000 units/ml of deoxyribonuclease (1 mg/ml). Store at 2-8°C.	≥ 1,000 units per vial	LK003170 LK003172	1 vi 5 vi	D2
PDS Kit, Inhibitor Vial Ovomucoid protease inhibitor and bovine serum albumin which is 0.22 micron filtered and lyophilized in autoclaved vials to contain 10 mg/ml each upon reconstitution with 32 ml of EBSS. Store at 2-8°C.	≥ 300 mg TRL inhibited per vial	LK003182	1 vi	OI-BSA
PDS Kit, EBSS Vial Earle's balanced salt solution (EBSS) as supplied in the Papain Dissociation System	N/A	LK003188	1 vi	EBSS



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# **Pepsin**

Source: Porcine Stomach

I.U.B.: 3.4.23.1 CAS Number: 9001-75-6

Pepsin is an acidic protease. Its inactive zymogen precursor, pepsinogen, is produced in the stomach mucosa. There are several pepsins designated A, B, C, and D. Pepsin A, the major component, has a molecular weight of 35 kDa and an optimum pH of approximately 1.0 for substrates such as casein or hemoglobin if the substrate is native protein. Pepsin cleaves proteins preferentially at carboxylic groups of aromatic amino acids such as phenylalanine and tyrosine. It will not cleave at bonds containing valine, alanine or glycine. Pepsin is assayed based on the method of Anson, *J. Gen. Physiol.*, 22, 79 (1938) using hemoglobin as the substrate. Pepsin is unstable above pH 6.

**Stability/Storage:** Pepsin is stable for 1-2 years at 2-8°C.

**Unit Definition**: One unit releases 0.001  $A_{280}$  as TCA soluble hydrolysis products from denatured hemoglobin per minute at 37°C. One FIP Unit, expressed as micromoles of tyrosine equivalents liberated per minute at 25°C, can be calculated as follows: 1 Worthington unit x 0.0071 = FIP Units.

Pepsin A PM

Two times crystallized from dilute alcohol.	≥ 2,500 units	LS003319	1 gm
A lyophilized powder.	per mg dry	LS003317	10 gm
Store at 2-8°C.	weight	LS003322	Bulk



Choose from a wide range of high quality enzymes for a variety of life science research applications.

Worthington Protease Products, Specifications and Applications Table								
Enzyme	Specificity	Molecular Weight KDa	pH Optimum	Extinction Coefficient E1%, 280nm	Common Substrates	Activators	Inhibitors	Product Code/ Applications
Partially Pur	ified for Tissue D	issociation	and Proteir	n Digestion				,
Collagenase	-Pro-X-†-Gly-Pro-Y- X = neutral Y = nonspecific	68-130	6.3-7.5	13.20 (CoIH, Theoretical) 13.40 (CoIG, Theoretical)	Collagen FALGPA Wünsch	Ca <sup>2+</sup> , Zn <sup>2+</sup>	α2-macroglobulin Cysteine, histidine DTT, 2-mercapto EDTA, EGTA Hg <sup>2+</sup> & other heavy metal ions <i>o</i> -phenanthroline	See page 14 for Collagenase products Tissue dissociation/ Primary cell isolation applications (see Tissue Dissociation Guide for specific references)
Elastase	Elastin, -X-†-Y- X = uncharged, nonaromatic Y = nonspecific	25.9	8.0-8.5	21.8 (Theoretical)	Casein Denatured collagen Elastin, Fibrin Suc-Ala3-NA	None required	α-antitrypsin DFP α2-macroglobulin PMSF	ES/ESL, suspension/ lyo powder, p. 28 Tissue Dissociation/ Primary cell isolation applications (see Tissue Dissocia- tion Guide for specific references)
Neutral Protease (Dispase®)	-X-†-Leu/Phe-†-Y- X/Y = nonspecific	36.0	5.9-7.0	13.96 (Theoretical)	BAEE Casein	$Ca^{2+}$ , $Mg^{2+}$ , $Mn^{2+}$ , $Fe^{2+}$ , and $Ai^{3+}$	EDTA, EGTA Hg <sup>2+</sup> & other heavy metal ions <i>o</i> -phenanthroline	NPRO/NPRO2, p. 44 Tissue Dissociation/ Primary cell isolation and cell harvesting applications (see Tissue Dissociation Guide for specific references)
Papain	-X-†-Y- X = nonspecific but Arg, Lys and Phe preferred Y = nonspecific	23.0	6.0-7.0	22.88 (Theoretical)	BAEE	Cysteine EDTA Reducing agents GSH, NBS	AEBSF, Antipain Cystatin, Leupeptin α2-macroglobulin Hg <sup>2+</sup> & other heavy metal ions DFP, PMSF TLCK, TPCK, E-64	PAP/PAPL, suspension/lyo powder, p. 46 Neural tissue dissociation/ primary cell isolation applications (see Tissue Dissociation Guide for specific references) Antibody cleavage RBC modification
Pepsin	-X-†-Y- X = nonspecific but aromatic & hydro- phobic preferred Y ≠ Ala, Gly, Val	34.6	1.0-4.0 unstable ≥5	14.39 (Theoretical)	Casein Hemoglobin	None required	Pepstatin A Diazoketones Epoxides	PM, p.50 Collagen bioprocessing/ purification Antibody cleavage
Proteinase K	-X-†-Y- X = nonspecific but aliphatic, aromatic & hydrophobic preferred Y = nonspecific	28.9	7.5-12	12.6 (Theoretical)	Casein Hemoglobin Keratin	Ca <sup>2+</sup> Active in 0.5- 1% SDS	DFP EGTA PMSF	PROK, PROKS, p. 59 DNA/RNA purification
Trypsin	-X-†-Y- X = Arg, Lys Y = nonspecific	23.8	7.5-8.5	14.3	BAEE Casein TAME	Ca <sup>2+</sup> Lanthanide	Aprotinin, Benzamidine DFP, EDTA, Leupeptin α2-macroglobulin PMSF, TLCK Trypsin Inhibitors (LBI, OI, SI/SIC)	See page 66 for Trypsin products Protein Digestion/ Sequencing (purified) Tissue dissociation/ Primary cell isolation applications (see Tissue Dissociation Guide for specific references)

# **Worthington Protease Products, Specifications and Applications Table**

Enzyme	Specificity	Molecular Weight KDa	pH Optimum	Extinction Coefficient E1%, 280nm	Common Substrates	Activators	Inhibitors	Product Code/ Applications
Proteases For	Protein Seque	ncing						
Carboxy- peptidase B	H2-N-Rn-Y-†-X- COOH X = basic amino acids (Arg, Lys, Orn) Y = nonspecific	34.3	7.0-9.0	21.4 (Folk 1971)	Hippuryl-L- arginine	None required	EDTA Hg <sup>2+</sup> & other heavy metal ions EDTA, EGTA <i>o</i> -phenanthroline	COBC/COBPMS, p.4 Sequence analysis by successive cleavage of C-terminal basic amino acids Insulin production
Carboxy- peptidase Y	H2-N-Rn-Y-†-X- C00H X ,Y= non-specific, prefers aromatic	64.0	4.5-6.0	15.0 (Hayashi <i>et al.</i> 1973, and Kuhn <i>et al.</i> 1973)	ATEE Bz-Phe-Ala-Leu Z-Phe-Ala	None required	APCK, Aprotinin DFP 4-Hydroxymercu- ribenzoate PMSF	COY, p. 5 C-terminal sequencing & Modification/labeling of peptides and proteins
Chymotrypsin TLCK treated	-X-†-Y- X = aromatic Y = nonspecific	25.6	7.8-8.0	20.57 (Theoretical)	ATEE BTEE	None required	α-antitrypsin Aprotinin DFP, PMSF, TPCK α2-macroglobulin	CDSEQ, CDTLCK, p. 10 Sequence analysis Peptide synthesis, mapping/finger- printing
Endo-Arg-C (Clostripain)	-Arg-†-Y- Y = nonspecific	53	7.4-7.8	16.57 (Theoretical)	BAEE	Ca <sup>2+</sup> Reducing agents	EDTA, TLCK, Tris Hg <sup>2+</sup> & other heavy metal ions	CPSEQ, CP, p.12 Peptide mapping & synthesis Sequence analysis Hydrolysis/ condensation of amide bonds
Endo-Glu-C (Staph. Protease V8)	-Glu-†-Y- (NH4 buffers pH 4, 7.8) -Asp-†-Y- (PO4 buffer pH 7.8)	27.0	4.0 & 7.8	4.26 (Houmard 1976)	Casein Z-Phe-Leu-Glu- 4NA	None required	DFP F-, CI-, Br-, CH3C00- N03- α2-macroglobulin	STSEQ, STAP, p. 58 Peptide mapping & sequence analysis
SequENZ® Trypsin, Sequencing Grade, Modified							Annekinin	TRSEQZ, Modified Sequencing Grade, p. 66 chemically modified to reduce autolysis Peptide mapping & sequence analysis Cleavage fusion proteins
Trypsin, Sequencing Grade, Native	-X-†-Y- X = Arg, Lys Y = nonspecific	X = Arg, Lys 23.8 7.5	7.5-8.5	7.5-8.5 14.3	BAEE Casein TAME	Ca <sup>2+</sup> Lanthanide	Aprotinin, Benzamidine DFP, EDTA, Leupeptin α2-macroglobulin PMSF, TLCK Trypsin Inhibitors (egg white, lima bean.	TRSEQII, Sequencing Grade, Native, p. 67 Peptide mapping & sequence analysis Cleavage fusion proteins
Trypsin, TPCK Treated							pancreatic, soybean)	TRTPCK, TPCK Treated, p. 67 Peptide mapping & sequence analysis Cleavage fusion proteins

# Protease, Staphylococcus aureus (Endoproteinase Glu-C)

Source: Staphylococcus aureus V8

Store at 2-8°C.

#### I.U.B.: 3.4.21.19 CAS Number: 66676-43-5

Protease *Staphylococcus aureus* V8 (Endoproteinase-Glu-C) specifically cleaves peptide bonds on the COOH-terminal side of either aspartic or glutamic acids. In the presence of ammonium, the enzyme specificity is limited to glutamic sites. It has a molecular weight of 27 kDa and optimum pH of 4.0 and 7.8 with hemoglobin as the substrate. Protease *Staphylococcus aureus* V8 is inhibited by diisopropylfluorophosphate and monovalent anions such as F<sup>-</sup>, Cl<sup>-</sup>, CH<sub>3</sub>COO-and NO<sub>3</sub><sup>-</sup>. Enzyme activity is determined by the casein digestion assay described by Drapeau, *Methods Enzymol.*, *45*, 469 (1976).

**Stability/Storage:** Autolysis occurs at temperatures greater than 40°C. The enzyme is fully active in USP 0.2% SDS. Stable for 12 months at 2-8°C.

**Unit Definition**: One unit causes a change of 0.001 A<sub>280</sub> nm per minute at 37°C, pH 7.8 using casein as the substrate.

Protease, S. aureus Sequencing Grade Chromatographically purified according to Drapeau, et al., J. Biol. Chem., 247, 6720 (1972). Supplied in vials containing 10 μg or 50 μg lyophilized powder for protein sequencing applications. Store at 2-8°C.	≥ 500 units per mg dry weight	LS02126 LS02128 LS02129	5x10 ug 5x50 ug Bulk	STSEQ
Protease, S. aureus (Endoproteinase Gluchromatographically purified according to Drapeau, G., Boily, Y., and Houmard, J., J. Biol. Chem., 247, 6720 (1972).  A lyophilized powder.	> 500 units per mg dry weight	LS003608 LS003605 LS003606	1 mg 5 mg Bulk	STAP



Worthington employees take pride in developing the highest quality enzymes.

#### **Proteinase K**

Source: Tritirachium album limber

I.U.B.: 3.4.21.64 CAS Number: 39450-01-6

Proteinase K (PROK) is a serine endopeptidase with a broad spectrum of action, isolated from the fungus *Tritirachium album limber*. Worthington Proteinase K is supplied as a highly purified lyophilized powder. It is tested to be free of DNase and RNase.

#### Characteristics of Proteinase K from Tritirachium album limber:

Molecular weight: 28.9 kDa. Extinction Coefficient: 14.2

pH Optimum: Stable over a wide pH range: 4.0-12.5, optimum pH 7.5-8.0, using denatured hemoglobin as substrate.

**Stability:** Although calcium ions do not affect the enzyme activity, they do protect PROK against autolysis and increase thermal stability when present at a concentration of 1 - 5 µmoles. An interesting characteristic of PROK is that it retains its activity in the presence of sodium dodecyl sulphate (SDS) or urea. (0.5 - 1% SDS and 1 - 4 M urea). Raising the temperature of the reaction from 37°C to 50 - 60°C can increase the activity several folds. A special feature of PROK is its ability to digest native proteins, thereby inactivating enzymes such as DNase and RNase without recourse to a denaturation process.

PROK is inactivated by diisopropyl fluorophosphate (DFP) or phenyl methane sulphonyl fluoride (PMSF). Chelating agents such as citrate and EDTA have no affect on the enzyme activity. PROK can also be inactivated by heating above 65°C for 15-20 minutes or by extraction with phenol/chloroform.

**Storage:** The lyophilized powder is stable for  $\geq$  1 year at 2-8°C. Solutions in 50 mM Tris-HCl, pH 8.0 with 1 mM CaCl<sub>2</sub> are stable for months at 2-8°C. Store at 2-8°C.

**Unit Definition**: One unit releases one micromole of Folin positive amino acids per minute, measured as tyrosine, at 37°C, pH 7.5, using urea denatured hemoglobin as the substrate.

Specificity: In addition to cleavage of peptide bonds, it is able to catalyze peptide amide hydrolysis.

**Application:** The recommended working concentration for PROK is 0.05-1 mg/ml. PROK is very useful in the isolation of highly native, undamaged DNAs or RNAs, since most microbial or mammalian DNases and RNases are rapidly inactivated by the enzyme, particularly in the presence of 0.2 - 1% SDS.

Proteinase K A lyophilized powder. Purified to remove DNase and RNase. Store at 2-8°C.	≥ 20 units per mg dry weight	LS004220 LS004222 LS004224 LS004226	25 mg 100 mg 1 gm Bulk	PROK
Proteinase K, Solution, 20mg/ml				PROKS
A concentrated, ready to use liquid	≥ 400 units	LS004240	5 ml	
formulation. Proteinase K prepared	per milliliter	LS004242	25 ml	
at 20 mg/ml in 10 mM Tris-HCl, 1 mM calcium acetate, pH 7.5 containing 50% glycerol. DNase and RNase free. Store at -20°C		LS004244	Bulk	
REQUIRES SPECIAL SHIPPING: ICE PACK	ζ.			

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#### **Trypsin**

Source: Bovine Pancreas

I.U.B.: 3.4.21.4 CAS Number: 9002-07-7

Trypsin is a pancreatic serine protease with substrate specificity based upon positively charged lysine and arginine side chains. It is derived from a 34 kDa inactive precursor zymogen, trypsinogen, after enzymatic removal of an N-terminal 6-amino acid leader sequence resulting in the 23.8 kDa trypsin molecule. The optimum pH is 8.0. Trypsin is inhibited by organophosphorus compounds such as diisopropylfluorophosphate and natural inhibitors from pancreas. Soybean, lima bean, and egg white are also sources of natural inhibitors. Trypsin cleaves amide and ester bonds of Arg and Lys. The Worthington Sequencing Grade Trypsin has been further purified to remove trace contaminating proteases and autolysis products which could interfere in trypsin digestion experiments, and exhibits a single band on SDS PAGE.

**Uses:** For tissue culture work, Worthington trypsin, Codes: TRL, TRLS, TRLVMF and TRTVMF have been used by many researchers. Product Codes: TRSEQZ, TRSEQII and TRTPCK are typically used for protein sequencing, mapping and structure studies. Worthington modified sequencing grade trypsin, Product Code: TRSEQZ, is subjected to extensive purification to remove contaminating proteases and tryptic autolysis by-products which could affect the specificity of the digestion process. Subsequently, the enzyme is chemically modified to minimize the autolysis process as well as increase the stability. The modified trypsin is processed further to remove residual autodegradation products. The specificity of the enzyme is routinely checked after the chemical modification.

**Stability/Storage:** Most grades of Worthington trypsin are stable for 2-3 years when stored at 2-8°C. Protect from moisture.

**Unit Definition**: TAME Unit: One Unit hydrolyzes 1 micromole of *p*-toluene-sulfonyl-L-arginine methyl ester (TAME) per minute at 25°C, pH 8.2, in the presence of 10 mM calcium.

One TAME Unit = 19.2 USP/NF units = 57.5 BAEE units.

**Technical Notes**: The Virus and Mycoplasma Free trypsin (Code: TRTVMF) has been filtered through an 0.22 micron pore size membrane, lyophilized, subjected to gamma irradiation, and tested for virus and mycoplasma.

Worthington certifies that all lots of Trypsin products are subjected to a pH of less than 3.0 for greater than five (5) hours during processing.

SequENZ® Trypsin, Modified, TRSEQZ

4 x 25 µg

1 mg

Bulk

4 x 100 µg

**Sequencing Grade** ≥ 150 Units Trypsin, treated with LS02120 L-(tosylamido-2-phenyl) ethyl LS02122 per mg protein chloromethyl ketone to inhibit (≥ 8,625 LS02123 contaminating chymotryptic activity, BAEE/2875 LS02124 chemically modified to promote stability USP/NF units and further purified to remove autolysis per mg protein) fragments, resulting in a highly stable trypsin product resistant to autolysis while retaining specificity. Store at -20°C PROTECT FROM MOISTURE.

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Name	Activity	Catalog Number	Package	Code
Frypsin (Continued)				
Trypsin, Purified, Sequencing Grade II Bovine trypsin that has been treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity and extensively purified to remove autolysis products. Supplied as a lyophilized powder. Store at -20°C. PROTECT FROM MOISTURE. REQUIRES SPECIAL SHIPPING: ICE PACK	≥ 150 Units per mg protein (≥ 8,625 BAEE/2875 USP/NF units per mg protein)	LS02115 LS02117 LS02119 LS02118	4 x 25 μg 4 x 100 μg 1 mg Bulk	TRSEQII
Trypsin, TPCK Treated A chromatographically purified, diafiltered, lyophilized powder that has been treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity (Kostka and Carpenter, <i>J. Biol. Chem.</i> 239, 1799, 1964. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 180 Units per mg protein (≥ 10,350 BAEE/3,450 USP/NF u/mg protein)	LS003740 LS003741 LS003744 LS003742	100 mg 500 mg 1 gm Bulk	TRTPCK
<b>Trypsin 3X</b> Supplied as a chromatographically purified, diafiltered and lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 180 Units per mg protein (≥ 10,350 BAEE/3,450 USP/NF u/mg protein)	LS003708 LS003707 LS003709	100 mg 1 gm Bulk	TRL3
<b>Trypsin 2X</b> Supplied as a dialyzed and lyophilized powder. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 180 Units per mg protein (≥ 10,350 BAEE/3,450 USP/NF u/mg protein)	LS003702 LS003703 LS003704 LS003706	100 mg 1 gm 10 gm Bulk	TRL

Name	Activity	Catalog Number	Package	Code
Trypsin (Continued)				
Trypsin, 0.22μ Filtered Trypsin chromatographically purified, diafiltered, (Code TRL3) filtered through a 0.22 micron pore size membrane and lyophilized in sterile vials. This product is not tested for pyrogenicity. Store at 2-8°C. PROTECT FROM MOISTURE.	≥ 180 Units per mg protein (≥ 10,350 BAEE/3,450 USP/NF u/mg protein)	LS003736 LS003734 LS003738	50 mg 5 x 50 mg Bulk	TRLS
<b>Trypsin 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 2 ml of HBSS yields a solution of 500 μg/ml of trypsin, Code: TRL Suitable for cell isolation and culture applications at 2-8°C.		LK003220 LK003225	1 vi 5 vi	TRLSNK
Trypsin, Sterile, Irradiated Chromatographically purified (Code: TRL), lyophilized, irradiated and tested for the absence of mycoplasma and extraneous virus according to 9 CFR113.53c. Each vial is filled to contain ≥ 100 mg. Store at 2-8°C.	≥ 180 Units per mg protein (≥ 10,350 BAEE/3,450 USP/NF u/mg protein)	LS004454 LS004452	100 mg 5 x 100 mg	TRLVMF
Trypsin, TPCK-Treated, Irradiated Chromatographically purified trypsin treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotryptic activity according to (Kostka and Carpenter, <i>J. Biol. Chem. 239</i> , 1799, 1964), Code: TRTPCK, lyophilized, irradiated and tested for the absence of mycoplasma and extraneous virus according to 9 CFR 113.53c. Each vial is filled to contain ≥ 100 m Store at 2-8°C.	≥ 180 Units per mg protein (≥ 10,350 BAEE/3,450 USP/NF u/mg protein)	LS003750 LS003752	100 mg 5 x 100 mg	TRTVMF

PROTECT FROM MOISTURE.

# **Trypsin Inhibitors**

**CAS Number: 9035-81-8** 

**Lima Bean Inhibitor**: Lima bean trypsin inhibitor, which inhibits bovine as well as human trypsin and plasmin, acts upon both trypsin and chymotrypsin by forming equimolar complexes. Lima bean inhibitors may be chromatographically separated into as many as six variants. Jones *et al.*, *Biochem.*, *2*, 66, (1963) characterized four of them. All have similar but not identical amino acid composition, contain six or seven disulfide bonds and lack methionine and tryptophan. Molecular weights vary between 8 kDa and 10 kDa.

Stability/Storage: The lima bean inhibitor is stable 1-2 years at 2-8°C.

**Ovomucoid**: Ovomucoids are the glycoprotein protease inhibitors of avian egg white. There are several protease inhibitors in egg white. The Worthington product is that described by Lineweaver and Murray, *J. Biol. Chem., 171*, 565 (1947). It has a molecular weight of approximately 28 kDa.

**Stability/Storage:** Ovomucoid is stable 1- 2 years when stored at 2-8°C.

**Soybean Inhibitor**: The soybean trypsin inhibitor was first crystallized by Kunitz in 1945 and is one of several such inhibitors found in soybeans. Its molecular weight is 21.5 ± 0.8 kDa and the optimum pH is 7.0. Soybean inhibitor inhibits trypsin mole-for-mole and to a lesser extent chymotrypsin.

**Stability/Storage:** The soybean inhibitor is stable for 1-2 years at 2-8°C.

**Unit Definition**: The activity of the inhibitors is expressed as the amount of twice crystallized trypsin (Worthington Code: TRL) inhibited per milligram of inhibitor. 1 mg TRL ≥ 180 TAME units, 10,350 BAEE units, 3,450 USP/NF units.

Trypsin Inhibitor, Lima Bean Animal Free				LBI
Fraction III of the preparation described by Fraenkel-Conrat <i>et al., Arch. Biochem. Biophys., 37,</i> 393 (1952). Supplied as a dialyzed, lyophilized powder. Store at 2-8°C.	1 mg inhibits ≥ 2.2 mg trypsin, Code: TRL	LS002829 LS002830 LS002831	100 mg 1 gm Bulk	PNIMAY PREE
<b>Trypsin Inhbitor, Ovomucoid</b> Mucoprotein and antitryptic factor of egg white described by Lineweaver and Murray, <i>J. Biol. Chem.</i> , <i>171</i> , 565 (1947). A dialyzed, dried powder. Store at 2-8°C.	1 mg inhibits ≥ 1.2 mg trypsin, Code: TRL	LS003085 LS003087 LS003086 LS003089	500 mg 1 gm 2 gm Bulk	OI
Trypsin Inhibitor, Soybean, Purified				SI
Animal Free Chromatographically purified. A dialyzed, lyophilized powder. Purity checked using SDS PAGE. Store at 2-8°C.	1 mg inhibits ≥ 1.2 mg trypsin, Code: TRL	LS003570 LS003571 LS003573	100 mg 1 gm Bulk	, NIMA,
Trypsin Inhibitor, Soybean Animal Free				SIC
Partially purified by methods developed at Worthington. A diafiltered, lyophilized powder.	1 mg inhibits ≥ 0.75 mg trypsin,	LS003587 LS003589 LS003590	1 gm 10 gm Bulk	FREE

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Payment terms are Net 30 Days, F.O.B. Origin, Lakewood, New Jersey USA, payable in US dollars. All checks must be drawn on a US bank or payment made by wire transfer. Past due accounts may be charged a 1.5% per month late payment fee.

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# **Quantity Discounts**

Quantity	Price
1 to 4	List Price
5 to 9	5% off list
10 to 19	10% off list
20 or more	15% off list
Bulk	Inquire

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For orders of greater than 25 packages, or orders of material packed in bulk, contact your representative or the Bulk Sales Office for special pricing consideration. Standing orders may also qualify for discounts. We welcome long-term use projections for which we can consider special rates. Large institutional buyers should contact their representative regarding special purchasing agreements.

# **Bulk, Contract/Custom & OEM Enzyme Purchasing**

As a primary manufacturer, Worthington can supply products in a wide range of purity and activity specifications and in large-scale bulk quantities at substantial discounts. In addition, we welcome inquiries for contract and custom manufacturing, custom analysis, and special packaging for OEM applications. Several products are listed as Bulk Only in this catalog due to limited availability. For more information on our services, see page 102. Please contact Customer Service or our Bulk Sales Office to discuss your specific requirements.

Complete Standard Terms and Conditions of Sale available on our website.

Phone: 800.445.9603 • 732.942.1660 • Fax: 800.368.3108 • 732.942.9270 Worthington-Biochem.com ISO9001 Certified

# **Technical Service**

Available 8:00 AM to 5:00 PM Eastern Time Monday through Friday. We can be contacted 24 hours a day by fax, e-mail or through our website.

Worthington makes the products we sell and welcome your questions and suggestions. Because we are a primary manufacturer we have ready access to all production and quality control records of our products by lot number.

Our years of experience in enzyme purification put us in a position to assist individual researchers with special needs. We frequently do customized preparations of entirely new products. We can make modifications of a regular production procedure on a custom basis. Furthermore, our quality control department can do special testing if needed.



Need help with protocols? Ask a representative how we can help update you with our latest technical tools.

# **Online Sampling Program**

Our position as the principal manufacturer of research grade collagenase makes possible our Collagenase Sampling Program. Under the program, we provide 100 mg samples of up to three different lots of collagenase for evaluation in your own cell isolation systems. A period of 60 days is allowed for your evaluation of these samples. 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, specify the lot desired when ordering. The only requirement, once a suitable lot of collagenase is found, is that you purchase a minimum of 3 grams of the material. **There is no charge for participating in the Collagenase Sampling Program.** 

There is no charge for participating in the Collagenase Sampling Program.

Contact your representative or our Technical Service group for more information: techservice@Worthington-Biochem.com.

# **Collagenase Lot Selection Tool**

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 on 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 and e-mail to assist with collagenase or any other products.

Phone: 800.445.9603 • 732.942.1660 • Fax: 800.368.3108 • 732.942.9270 Worthington-Biochem.com ISO9001 Certified

# ISO 9001 Certified Quality Management System

Worthington Biochemical Corporation is company-wide ISO9001 certified and operates according to GMP guidelines. Our initial ISO assessment audit was performed by ANAB-accredited SGS US Testing Company, Systems & Services Certification in 2005 with continuous successful re-certifications.

# **Product Use**

All Worthington products are sold for manufacturing, research, and laboratory use only by properly trained and authorized personnel. Researchers and clinical laboratory personnel intending to use any of these products for medical investigation on humans are solely responsible for such use, and for compliance with the pertinent regulations of the United States Food & Drug Administration (USFDA) and other regulations. We do not assume liability for damages resulting from the use of these products or from their use in violation of patent or other rights.

# **U.S.D.A.** Certified Raw Materials

All products from animal sources are produced from starting materials of United States Department of Agriculture (USDA) or equivalent approved origin, collected in USDA or equivalent approved facilities, inspected to be free of disease and suitable for exportation. Certificates of Origin are available upon request.

# **Animal Free Products**

Several Animal Free (AF) nucleases, proteases and other products are also available to eliminate BSE/TSE and mammalian viral risks. Please inquire. All animal free products are designated with this symbol for ease of use.

# **Product Returns**

Authorization for any product return must be obtained from Worthington Biochemical Corporation (Customer Service Department), or its authorized representative, prior to the return of product. This authorization is required to insure the proper return of material and, if applicable, the correct issuance of credit. There is no provision for credit of misused, improperly stored or outdated material. Product(s) must be returned in the same condition as received within 30 days of the original shipment by Worthington Biochemical Corporation. A restocking fee may be charged.

Complete Standard Terms and Conditions of Sale available on our website.

# **Online Resources**

# Hundreds of pages of technical, product and reference information are available online at: Worthington-Biochem.com

Worthington Enzyme Manual

Tissue Dissociation Guide

Catalog and Price List

Online Collagenase Lot Selection Tool

# **Additional Features**

Complete Searchability

Current Collagenase Lot Availability and Activities

Online Ordering and Technical Service

Updated Announcements and Exhibit Schedules

International Distributor Listing

Useful Links

Visit us at: facebook Linked in



Addressing your individual needs, we value every customer interaction. Let us know how we are doing.

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# **Brazil:**

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# Sinapse Biotecnologia Ltda

Website: www.sinapsebiotechnologia.com.br

#### Canada:

Canadian Customers may contact us directly:

# **Worthington Biochemcal Corporation**

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#### Please visit: Worthington-Biochem.com

For territories not listed or contact International Sales,

Email: international@Worthington-Biochem.com

# Our Driving Force is Quality

The energy that drives all Worthington employees is the deep commitment to consistently produce superior quality products while providing personal service.

Our ISO9001 certified enzyme manufacturing facility and proprietary processes are designed specifically for the life science community, bioprocessing and OEM/private label business partners. We are proud to be a primary producer with over 90% of our products manufactured at our core facility located in Lakewood, New Jersey, USA.

# Value Verified



ISO9001 Certified Quality Management System

All Processes Fully Documented and Traceable from Raw Materials through Final Shipment



Primary Producer For Biotech, Life Science Research, Diagnostic, Biopharmaceutical and Bioprocessing

Respected Manufacturer of High Quality, Lot-to-Lot Consistent Enzymes for Over 70 Years



Multi-Scale Extraction, Fermentation, Protein Purification, Lyophilization and Packaging Capabilities Produced Under GMP Guidelines
Internal Testing Capabilities include Enzyme, Protein, and Related Biochemical Analysis and Characterization



Animal-Free Certified and USDA Approved Animal-Sourced Products Suitable for Worldwide Exportation



Flexible and Responsive Production Scheduling
Sample Lots and Bulk Inventories Available for Immediate Shipment



Superior Customer Services and Technical Support — Before, During and After Sale

Volume Purchases, Bulk Packaging, Standing Orders, OEM/Supply Agreements, and Special Arrangements



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