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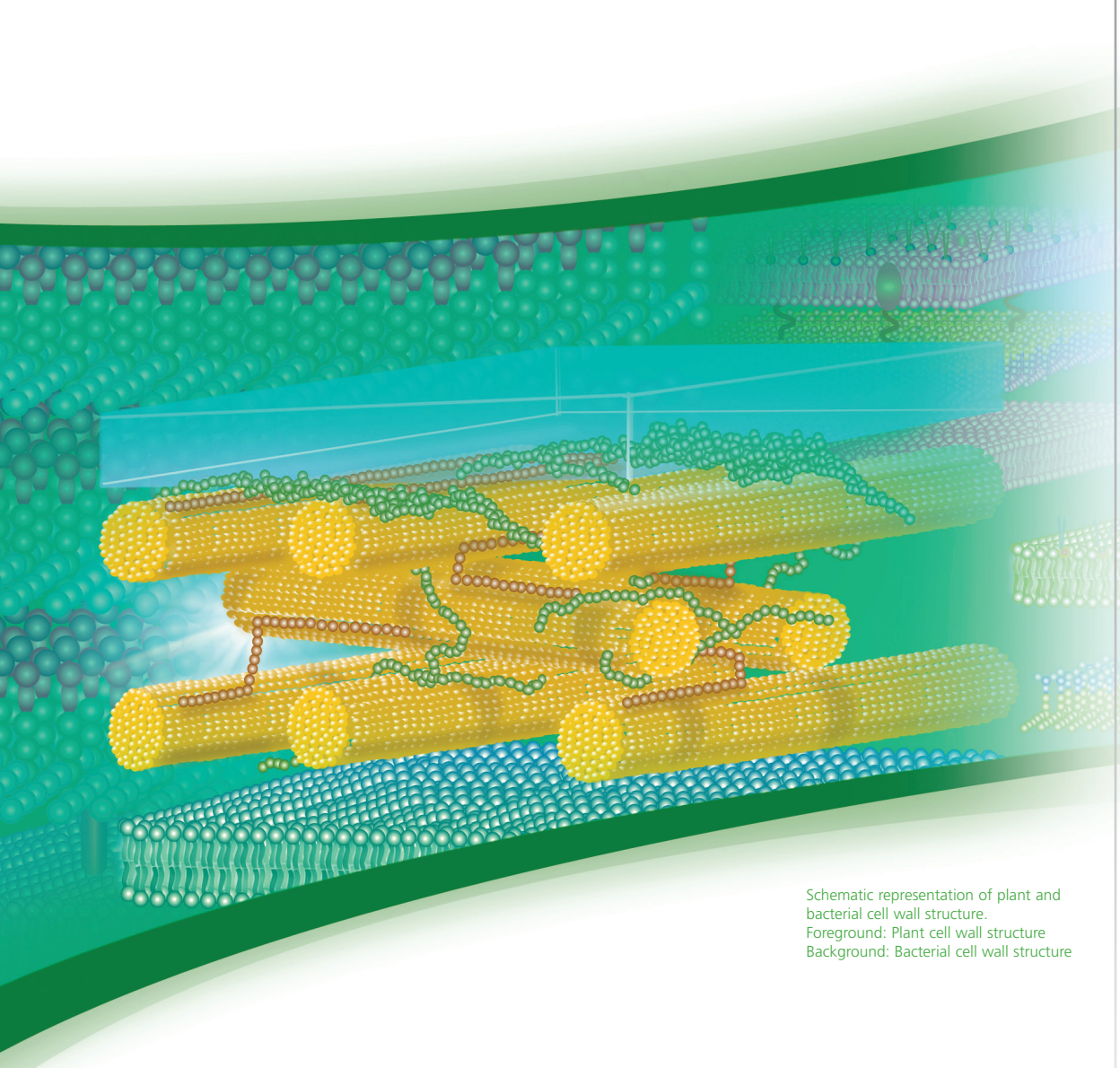
FOR LIFE SCIENCE RESEARCH

Issue 2, 2006

SIGMA



DETACHMENT OF CULTURED CELLS

LYSIS AND PROTOPLAST PREPARATION OF:
Yeast
Bacteria
Plant Cells

PERMEABILIZATION OF MAMMALIAN CELLS

MITOCHONDRIA ISOLATION

Schematic representation of plant and bacterial cell wall structure.
Foreground: Plant cell wall structure
Background: Bacterial cell wall structure

Enzymes for Cell Dissociation and Lysis

sigma-aldrich.com

SIGMA-ALDRICH

The Sigma Aldrich Web site offers several new tools to help fuel your metabolomics and nutrition research

The new Metabolomics Resource Center at:
sigma-aldrich.com/metpath

Sigma-Aldrich is proud of our continuing alliance with the International Union of Biochemistry and Molecular Biology. Together we produce, animate and publish the Nicholson Metabolic Pathway Charts, created and continually updated by Dr. Donald Nicholson. These classic resources can be downloaded from the Sigma-Aldrich Web site as PDF or GIF files at no charge. This site also features our metabolite libraries and kits for metabolite and dietary analysis.

The Nutrition Research Bioactive Nutrient Explorer at:
sigma-aldrich.com/nutrition

Nutrient analysis, chemoprevention, bioavailability and nutrient interactions are emerging as pathways to understanding relationships between diet and health, disease and metabolism. The Bioactive Nutrient Explorer is designed to help you identify structurally related chemicals and locate compounds found in specific plant species.

For additional information on each enzyme refer to the Sigma-Aldrich Enzyme Explorer at:
sigma-aldrich.com/enzymexplorer

- Package Sizes, Prices and Availability
- Data Sheets
- Certificates of Analysis
- Assay Procedures
- MSDSs
- Related Products

For Hazard Information and other information please refer to the Sigma Biochemicals, Reagents and Kits for Life Science Research Catalog or sigma-aldrich.com

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FOR LIFE SCIENCE RESEARCH
Issue 2, 2006

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Table of Contents

Enzymes for Cell Detachment and Tissue Dissociation

Collagenase

Collagenase cleaves the peptide bonds in native, triple-helical collagen. Because of its unique ability to hydrolyze native collagen, it is widely used in isolation of cells from animal tissue. Collagenases occur in a variety of microorganisms and many different animal cells.[1] The most potent collagenase is the "crude" collagenase secreted by the anaerobic bacterium *Clostridium histolyticum*. *C. histolyticum* collagenases have molecular weights from 68,000 to 125,000 Da and are metalloproteinases that require zinc and calcium.

The original 1953 fermentation and purification process described by MacLennan, Mandl, and Howes[2] was first adopted by Sigma-Aldrich and eventually improved upon for higher activity products. "Crude" collagenase refers to the fact that the material is actually a mixture of several different enzymes in addition to collagenase that act together to break down tissue. It is now known that two forms of the collagenase enzyme are present.[3,4] With a few exceptions different commercial collagenases are all made from *C. histolyticum,* or are recombinant versions where *E. coli* expresses a gene cloned from *C. histolyticum.* We provide a lot reservation service. You may purchase small quantities from one or more lots and reserve larger quantities until your evaluation is complete.

The different collagenase products in the tables were developed by Sigma-Aldrich because they digested different types of tissue (muscle, pancreas, heart, adipose) better than others. Besides meeting enzyme activity specifications, every lot of many Sigma-Aldrich collagenase products must pass digestion tests with various tissues from rats. Products that are also described as "cell culture tested" have undergone additional testing with mammalian cell lines to verify that they are not cytotoxic.

Sterile-filtered (0.2 µm) versions prepared from some of the more popular collagenase products are also available.

Sigma-Aldrich's purified collagenase products have only trace amounts of caseinase (proteolytic) or clostripain activities. The purified Type VII Collagenase is also offered in cell culture tested and sterile-filtered versions.

Enzymatic Assays for Collagenase

The isoforms I and II of purified collagenase differ in their specificities and relative activities on native collagen and synthetic substrates. These two collagenases can be distinguished by their preference for one of the two different substrates used in Sigma-Aldrich's assays. The Collagenase Digestive Unit (CDU) assay[10,11] measures predominantly the Collagenase I activity, which cleaves two of the three helical chains in the long, undenatured collagen protein. Collagenase II activity is measured by this enzyme's ability to cut a short synthetic peptide, N-[3-(2-Furyl)acryloyl)]-Leu-Gly-Pro-Ala (FALGPA, see Cat. No. F5135), in a second collagenase digestive assay.[12,13] Purified preparations of either isoform I or II have been shown to be less effective at digesting various types of collagen or mammalian tissue when compared to a mixture of both forms of the enzyme. Obviously the combination of true collagenase and the different native proteases, clostripain and aminopeptidases that have evolved in nature assist each other in digesting the collagen in different animal tissues. For tissue digestions the crude collagenase products have always been the most effective. Some researchers have tried mixtures of chromatographically purified collagenase with a protease such as trypsin or subtilisin to digest tissue.

In addition to the CDU and FALGPA assays for collagenase activities Sigma-Aldrich tests each product lot for caseinase,[14,15] clostripain and tryptic activities to evaluate the proteolytic activities in our collagenase products. The caseinase assay is the most important of the three for measuring the proteolytic activity that assists the digestion of animal tissue. Because the clostripain present in crude collagenase must be reduced (e.g., by treatment with dithiothreitol) in order to be active this enzyme probably contributes little to the tissue dissociation process in the laboratory. It is monitored because some researchers have reported that clostripain may be damaging or toxic.

Many collagenase products that meet enzymatic specifications are also application-tested with various tissues obtained from rats. Type II (C6885, C1764) and Type VIII (C2139) collagenase lots are tested for the ability to release adipose (fat) cells from rat epididymal fat pads.[5] Fat cells are then screened for metabolic activity by measuring glucose oxidation rates with and without insulin addition. Type IV (C5138, C1889) and Type VIII (C2139) lots have been tested for the ability to release viable cells from rat liver.[7] Type V (C9263, C2014), Type XI (C7657, C4785, C9407, and C9697) and Type S (C6079) collagenase lots must release intact islets of Langerhans from rat pancreas to pass their product test.[8]

Enzymes for Cell Detachment and Tissue Dissociation

Cat No.	Type	Specific activity (units per mg Solid) FALGPA	Neutral Protease	Applications, Comments
SIGMA BLEND™ COLLAGENASES				
C7926	Sigma Blend™ F	1.8-2.2	≤Σ10	Mostly purified collagenases in this blend
C8051	Sigma Blend™ H	1.1-1.5	20-50	Some native protease in this blend
C8176	Sigma Blend™ L	0.5-0.9	50-80	More native protease in this blend

Cat No.	Type	Specific activity (units per mg Solid) FALGPA	CDU	Applications, Comments
CRUDE, GENERAL USE				
C0130	Type I	0.25-1.0	>125	For general use
C1639	Type I-S	0.25-1.0	>125	Sterile-filtered from Type I (C0130)
C9891	Type IA	0.5-5.0	>125	For general use
C5894	Type IA-S	0.5-5.0	>125	Sterile-filtered from Type IA (C9891)
APPLICATION TESTED				
C2674	Type IA	0.5-2.0	>125	Cell culture tested from Type IA (C9891)
C9722	Type IA-S	0.5-3.0	>125	Sterile-filtered and cell culture tested from Type IA (C2674)
C6885	Type II	0.5-5.0	>125	For release of epididymal adipocytes (fat cells)
C1764	Type II-S	0.5-3.0	>125	Sterile-filtered from Type II (C6885)
C5138	Type IV	0.5-5.0	>125	For release of hepatocytes
C1889	Type IV-S	0.5-3.0	>125	Sterile-filtered from Type IV (C5138)
C2139	Type VIII	0.5-5.0	>125	For release of adipocytes and hepatocytes
C9263	Type V	1.0-3.0	>125	For release of pancreatic islets
C2014	Type V-S	1.0-3.0	>125	Sterile-filtered from Type V (C9263)
C7657	Type XI	2.0-5.0	>1200	For release of pancreatic islets;
C4785	Type XI-S	2.0-5.0	>1200	Sterile-filtered from Type XI (C7657)
C9407	Type XI	2.0-5.0	>1200	Cell culture tested from Type XI (C7657)
C9697	Type XI-S	2.0-5.0	>1200	Sterile-filtered from Type XI & cell culture tested (C9407)
HIGH-PURITY: CHROMATOGRAPHICALLY PURIFIED				
C0255	Type III	2-10	Min. 400	Substantially free of protease; may contain clostripain
C0773	Type VII	4-12	1000-3000	Substantially free of protease and clostripain
C2399	Type VII-S	4-12	1000-3000	Sterile-filtered from Type VII (C0773)
C2799	Type VII	4-12	1000-3000	Cell culture tested from Type VII (C0733)
C9572	Type VII-S	4-12	1000-3000	Sterile-filtered from Type VII and cell culture tested (C2799)

Crude Collagenase: Application Tested

Collagenase from *Clostridium histolyticum*

Clostridiopeptidase A
[9001-12-1] E.C. 3.4.24.3; EC No. 2325829

One **collagen digestion unit** liberates peptides from collagen equivalent in ninhydrin color to 1.0 µmole of leucine in 5 hr at pH 7.4 at 37 °C in the presence of calcium ions.

One **FALGPA hydrolysis unit** hydrolyzes 1.0 µmole of furylacryloyl-Leu-Gly-Pro-Ala per min at pH 7.5 at 25 °C in the presence of calcium ions.

One **neutral protease unit** hydrolyzes casein to produce color equivalent to 1.0 µmole of tyrosine per 5 hr at pH 7.5 at 37 °C.

One **clostripain unit** hydrolyzes 1.0 µmole of BAEE per min at pH 7.6 at 25 °C in the presence of DTT.

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-582-9

▸ release of physiologically active rat epididymal adipocytes tested, Type II, activity: >125 CDU/mg solid (CDU = collagen digestion units), activity: 0.5–5.0 FALGPA units/mg solid

Also contains clostripain, nonspecific neutral protease, and tryptic activities

References
1. Rodbell, M., *J. Biol. Chem.* **239**, 375 (1964)

-20°C

C6885-25MG	25 mg
C6885-100MG	100 mg
C6885-500MG	500 mg
C6885-1G	1 g
C6885-5G	5 g

▸ sterile-filtered, release of physiologically active rat epididymal adipocytes tested, Type II-S, activity: 0.5–3.0 FALGPA units/mg solid

lyophilized powder

Prepared from Type II (C6885)

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

References
1. Rodbell, M., *J. Biol. Chem.* **239**, 375 (1964)

-20°C

C1764-50MG	50 mg

▸ release of physiologically active rat hepatocytes tested, Type IV, activity: 0.5-5.0 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units)

Also contains clostripain, nonspecific neutral protease and tryptic activities.

References
1. Seglen, *Methods Cell Biol.* **13**, 29 (1976)

-20°C

C5138-25MG	25 mg
C5138-100MG	100 mg
C5138-500MG	500 mg
C5138-1G	1 g
C5138-5G	5 g

▸ sterile-filtered, release of physiologically active rat hepatocytes tested, Type IV-S, activity: 0.5-3.0 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units) lyophilized powder Prepared from Type IV (C5138)

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

References
1. Seglen, P.O., *Methods Cell Biol.* **13**, 29–83 (1976)

-20°C

C1889-50MG	50 mg

Crude Collagenase: Application Tested cont'd

- **release of rat epididymal adipocytes and hepatocytes tested (for methodology see Type II and Type IV), Type VIII, activity: 0.5–5.0 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units)**

Also contains clostripain, nonspecific neutral protease and tryptic activities.

–20°C

C2139-100MG	100 mg
C2139-500MG	500 mg
C2139-1G	1 g
C2139-5G	5 g

- **release of physiologically active rat pancreatic islets tested, Type V, activity: 1–3 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units)**

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

References

1. Lacy, P.E., Kostianovsky, M., *Diabetes* **16**, 35 (1967)

–20°C

C9263-25MG	25 mg
C9263-100MG	100 mg
C9263-500MG	500 mg
C9263-1G	1 g
C9263-5G	5 g

- **sterile-filtered, suitable for release of physiologically active rat pancreatic islets, Type V-S, activity: 1–3 FALGPA units/mg solid, activity: >125 CDU/mg solid, lyophilized powder**

Prepared from Type V (C9263)

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

–20°C

C2014-50MG	50 mg

- **release of physiologically active rat pancreatic islets tested, Type XI, activity: 2–5 FALGPA units/mg solid, activity: >1200 CDU/mg solid (CDU = collagen digestion units)**

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

References

1. Lacy, P.E., Kostianovsky, M., *Diabetes* **16**, 35 (1967)

–20°C

C7657-25MG	25 mg
C7657-100MG	100 mg
C7657-500MG	500 mg
C7657-1G	1 g
C7657-5G	5 g

- **sterile-filtered, release of physiologically active rat pancreatic islets tested, Type XI-S, activity: 2–5 FALGPA units/mg solid, activity: >1200 CDU/mg solid (CDU = collagen digestion units) lyophilized powder**

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

Prepared from Type XI (C7657)

–20°C

C4785-50MG	50 mg

- **powder, Suitable for the digestion and isolation of physiologically active pancreatic islet cells, cell culture tested Prepared from Type XI (C7657)**

Also contains clostripain, nonspecific neutral protease and tryptic activities.

–20°C

C9407-25MG	25 mg
C9407-100MG	100 mg
C9407-500MG	500 mg
C9407-1G	1 g
C9407-5G	5 g

- **lyophilized powder (from sterile-filtered solution), Suitable for digestion and isolation of physiologically active pancreatic islet cells, cell culture tested**

Prepared from Type XI (C9407).

Also contains clostripain, nonspecific neutral protease and tryptic activities.

–20°C

C9697-50MG	50 mg

Crude Collagenase for General Use

Collagenase from *Clostridium histolyticum*

Clostridiopeptidase A
[9001-12-1] E.C. 3.4.24.3; EC No. 2325829

One **collagen digestion unit** liberates peptides from collagen equivalent in ninhydrin color to 1.0 µmole of leucine in 5 hr at pH 7.4 at 37 °C in the presence of calcium ions.

One **FALGPA hydrolysis unit** hydrolyzes 1.0 µmole of furylacryloyl-Leu-Gly-Pro-Ala per min at pH 7.5 at 25 °C in the presence of calcium ions.

One **neutral protease unit** hydrolyzes casein to produce color equivalent to 1.0 µmole of tyrosine per 5 hr at pH 7.5 at 37 °C.

One **clostripain unit** hydrolyzes 1.0 µmole of BAEE per min at pH 7.6 at 25 °C in the presence of DTT.

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-582-9

- **for general use, Type I, activity: 0.25–1.0 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units), essentially salt-free, lyophilized powder**

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

Equivalent to first 40% ammonium sulfate fraction of Mandl, I., *et al.*, *J. Clin. Invest.*, **32**, 1323 (1953).

–20°C

C0130-100MG	100 mg
C0130-500MG	500 mg
C0130-1G	1 g
C0130-5G	5 g

- **sterile-filtered, for general use, Type I-S, activity: 0.25–1.0 FALGPA units/mg solid, activity: ≥125 CDU/mg solid lyophilized powder**

Prepared from Type I (C0130)

Also contains clostripain, nonspecific neutral protease and tryptic activities.

–20°C

C1639-50MG	50 mg

Enzymes for Cell Detachment and Tissue Dissociation

Crude Collagenase for General Use cont'd

Type IA, activity: 0.5-5.0 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units), essentially salt-free, lyophilized powder

For general use

Equivalent to first 40% ammonium sulfate fraction of Mandl, I., et al., *J. Clin. Invest.*, **32**, 1323 (1953).

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

Similar to Type I, but produced by Sigma.

–20°C

C9891-25MG	25 mg
C9891-100MG	100 mg
C9891-500MG	500 mg
C9891-1G	1 g
C9891-5G	5 g

sterile; sterile-filtered, Type IA-S, activity: 0.5–5.0 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units) lyophilized powder

Prepared from Type IA (C9891)

Also contains clostripain, nonspecific neutral protease and tryptic activities.

–20°C

C5894-50MG	50 mg

lyophilized powder, activity: 0.5–2.0 FALGPA units/mg solid, activity: >125 CDU/mg solid (CDU = collagen digestion units), cell culture tested

Also contains clostripain, nonspecific neutral protease and tryptic activities.

Crude

Type I-A

–20°C

C2674-100MG	100 mg
C2674-500MG	500 mg
C2674-1G	1 g

lyophilized powder (from sterile-filtered solution), cell culture tested

Prepared from Type IA (C2674).

Also contains clostripain, nonspecific neutral protease and tryptic activities.

–20°C

C9722-50MG	50 mg

Chromatographically Purified Collagenase

Collagenase from *Clostridium histolyticum*

Clostridiopeptidase A
[9001-12-1] E.C. 3.4.24.3; EC No. 2325829

One **collagen digestion unit** liberates peptides from collagen equivalent in ninhydrin color to 1.0 µmole of leucine in 5 hr at pH 7.4 at 37 °C in the presence of calcium ions.

One **FALGPA hydrolysis unit** hydrolyzes 1.0 µmole of furylacryloyl-Leu-Gly-Pro-Ala per min at pH 7.5 at 25 °C in the presence of calcium ions.

One **neutral protease unit** hydrolyzes casein to produce color equivalent to 1.0 µmole of tyrosine per 5 hr at pH 7.5 at 37 °C.

One **clostripain unit** hydrolyzes 1.0 µmole of BAEE per min at pH 7.6 at 25 °C in the presence of DTT.

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-582-9

purified by chromatography, Type III, activity: 2–10 FALGPA units/mg solid, activity: ≥400 CDU/mg solid (CDU = collagen digestion units), lyophilized powder

neutral protease.....<1 unit/mg solid
clostripain .....may contain trace amount

References

1. Mandl, I., et al., *J. Clin. Invest.* **32**, 1323 (1953)

–20°C

C0255-1.5KU	1,500 units
C0255-3KU	3,000 units
C0255-7.5KU	7,500 units

high purity, purified by chromatography, Type VII, activity: 4–12 FALGPA units/mg solid, lyophilized powder, activity: 1,000-3,000 CDU/mg solid (CDU = collagen digestion units)

Typically used with a neutral protease for tissue disruption.

Lyophilized powder containing calcium chloride

composition

Protein ~95% (biuret)
neutral protease and clostripain..... <1 unit/mg protein

–20°C

C0773-1.5KU	1,500 units
C0773-3KU	3,000 units
C0773-7.5KU	7,500 units
C0773-15KU	15,000 units
C0773-30KU	30,000 units
C0773-60KU	60,000 units

sterile-filtered, high purity, purified by chromatography, Type VII-S, activity: 4–12 FALGPA units/mg solid, activity: 1,000–3,000 CDU/mg solid (CDU = collagen digestion units)

lyophilized powder

Prepared from Type VII (C0773)

–20°C

C2399-1.5KU	1,500 units
C2399-3KU	3,000 units
C2399-7.5KU	7,500 units
C2399-15KU	15,000 units

powder, high purity, activity: 4–12 FALGPA units/mg solid, activity: 1000–3000 CDU/mg solid (CDU = collagen digestion units), cell culture tested purified by chromatography

Prepared from Type VII (C0773)

neutral protease and clostripain .....<1 unit/mg solid

–20°C

C2799-7.5KU	7,500 units
C2799-15KU	15,000 units

lyophilized powder (from sterile-filtered solution), high purity, cell culture tested

Prepared from Type VII (C2799)

–20°C

C9572-7.5KU	7,500 units
C9572-15KU	15,000 units

Crude Collagenase with proteolytic activity inhibitor

Collagenase + protease inhibitor

E.C. 3.4.24.3

R: 20/21/22-42/43 S: 26–36

activity: 2–5 FALGPA units/mg solid, activity: ≥1,000 CDU/mg solid (CDU = collagen digestion units), Suitable for isolation of rat pancreatic islet cells.

Selected lots of collagenase Type XI blended with protease inhibitor to limit the tryptic enzyme activities in pancreatic tissue. The formulation was developed through collaboration with several outside laboratories.

Also contains clostripain and nonspecific neutral protease activities.

Specifically formulated for pancreatic islet isolation.

–20°C DRY ICE

C6079-500MG	500 mg
C6079-1G	1 g
C6079-5G	5 g

Sigma Blend™ Collagenases

Collagenase from *Clostridium histolyticum*

Clostridiopeptidase A
[9001-12-1] E.C. 3.4.24.3; EC No. 2325829

One **collagen digestion unit** liberates peptides from collagen equivalent in ninhydrin color to 1.0 µmole of leucine in 5 hr at pH 7.4 at 37 °C in the presence of calcium ions.

One **FALGPA hydrolysis unit** hydrolyzes 1.0 µmole of furylacryloyl-Leu-Gly-Pro-Ala per min at pH 7.5 at 25 °C in the presence of calcium ions.

One **neutral protease unit** hydrolyzes casein to produce color equivalent to 1.0 µmole tyrosine per 5 hr at pH 7.5 at 37 °C.

One **clostripain unit** hydrolyzes 1.0 µmole of BAEE per min at pH 7.6 at 25 °C in the presence of DTT.

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-582-9

activity: 1.1–1.5 FALGPA units/mg solid, Sigma Blend Type H

neutral protease activity: 20–50 units/mg solid

–20°C

C8051-100MG	100 mg
C8051-500MG	500 mg
C8051-1G	1 g
C8051-5G	5 g

Sigma Blend Type L, activity: 0.5–0.9 FALGPA units/mg solid

neutral protease activity: 50–80 units/mg solid

–20°C

C8176-25MG	25 mg
C8176-100MG	100 mg
C8176-500MG	500 mg
C8176-1G	1 g
C8176-5G	5 g

Sigma Blend Type F, activity: 1.8–2.2 FALGPA units/mg solid

neutral protease activity: ≤10 units/mg solid

–20°C

C7926-25MG	25 mg
C7926-100MG	100 mg
C7926-500MG	500 mg
C7926-1G	1 g
C7926-5G	5 g

Collagenase Alternative

Accutase® solution

Special formulation that gently and rapidly dissociates tissues for cell isolation and propagation. This combines protease and collagenolytic activities which maximizes its versatility for cell detachment of adherent cells and tissue dissociation. Proven effective in detaching primary fibroblasts, endothelial cells, neurons, tumor cell lines, and insect cells. Performs exceptionally well in detaching cells for analysis of cell surface markers, virus growth assay, and flow cytometry as well as bioreactor scale-up. Does not contain mammalian or bacterial-derived products.

pH 6.8–7.8, sterile-filtered, cell culture tested

Prepared in Dulbecco's PBS (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8 g/L NaCl, and 1.15 g/L Na_2HPO_4) containing 0.5 mM EDTA•4Na and 3 mg/L Phenol Red. Refer to Product Data Sheet on the Web site for product usage information.

Features & Benefits

Ready-to-use sterile liquid for *in vitro* cell applications

–20°C

A6964-100ML	100 mL

Enzymes for Cell Detachment and Tissue Dissociation

Collagenase Trouble Shooting and References

Tissue Digestion—Dissociation by Collagenase

Problem	Cause	Solution	Ref.
Digestion is Poor	Inactive enzymes	Store collagenase cold and dry. Store collagenase solution in frozen aliquots	6
	Inadequate Ca^{++}	Include 5 mM Ca^{++} in collagenase solution	7
	Insufficient enzymes	Use more collagenase. Try Sigma Blends with more protease activity.	
Released Cells are Trapped	DNA released from broken cells	Reduce agitation	7
		Add DNase**	9
	Non-protein gums	Flush tissue well before digesting it***	7
		Use no Mg^{++} in digestion solution	7
		Add hyaluronidase with enzymes**	18
Cells are Killed	Excess protease	Reduce exposure to proteases* Add albumin or heated serum	
	pH changes	Use buffers (e.g., HEPES) instead of HCO_3^-. Check and re-adjust pH often	7
	Too little oxygen	Aerate digestion solution (air or O_2) Digest faster by using more enzymes	19
Cell Yield is Low	Enzyme balance	Use more collagenase*	
		Include elastase with collagenase**	20
	Adhesion factors	Perfuse tissue first to remove Ca^{++}****	7, 22
Many Cells are Damaged	Excessive protease	Use less protease*	21
		Add albumin or heated serum	
	Physical damage	Handle tissue and cells very gently	7, 22
New Lot Doesn't Work	Lot variation	Use fractionated Sigma blend collagenases (Sigma Catalog Nos. C7926, C8051 or C8176)*	

* The separately prepared collagenase and protease enzymes in the "Sigma Blend" products (Cat. No. C7926, C8051 or C8176) give reproducible control of how much of each is used.

** DNase will be inactivated by the shear of excessive stirring, and added enzymes may be digested by the neutral protease present in the collagenase.

*** Use EGTA (or EDTA) to remove Ca^{2+} and flush away microorganisms, then wash tissue with buffer to remove the chelating agent. Do not add EGTA or EDTA to the enzyme solutions.

Factors that Affect Tissue Digestion-Dissociation by Collagenase

Based on our own R and D and from discussions with customers it is clear that the way a particular tissue is dissected and prepared has a significant effect on the speed and efficiency of any tissue digestion-dissociation with collagenase. Differences in the ages of the tissue donors can also be a major source of variation over time. Make sure that calcium ions are present in the digestion buffers at 5 mM. Chelating agents EGTA and EDTA can severely inhibit collagenase activity by removing calcium ions required for enzyme stability and activity. β-mercaptoethanol,[16] cysteine[16] and 8-hydroxyquinoline-5-sulfonate[16] are other inhibiting substances. A new lot of collagenase with higher specific activity could cause excessive cell death at an established concentration. In that case use less collagenase and/or add BSA or serum (up to 0.5% and 5–10% respectively) to stabilize the cells during digestion.

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8. Lacy, P.E., and Kostianovsky, M., *Diabetes*, **16**, 35 (1967)
9. Buitrago, A., et al., *Biochem. Biophys. Res. Commun.*, **79**, 823 (1977)
10. Moore S., and Stein, W.H., *J. Biol. Chem.,* **176**, 367 (1948)
11. Sigma-Aldrich quality control test procedure
12. Van Wart, H.E., and Steinbrink, D.R., *Anal. Biochem.*, **113**, 356 (1981)
13. Sigma-Aldrich quality control test procedure
14. Anson, M.L., *J. Gen. Physiol.*, **22**, 79 (1938)
15. Sigma-Aldrich quality control test procedure
16. Seifter, S., et al., *J. Biol. Chem.*, **234**, 285 (1959)
17. Enzyme Handbook, D.Schomberg and M. Salzmann, Editors. Springer-Verlang, 1991, Vol. 5
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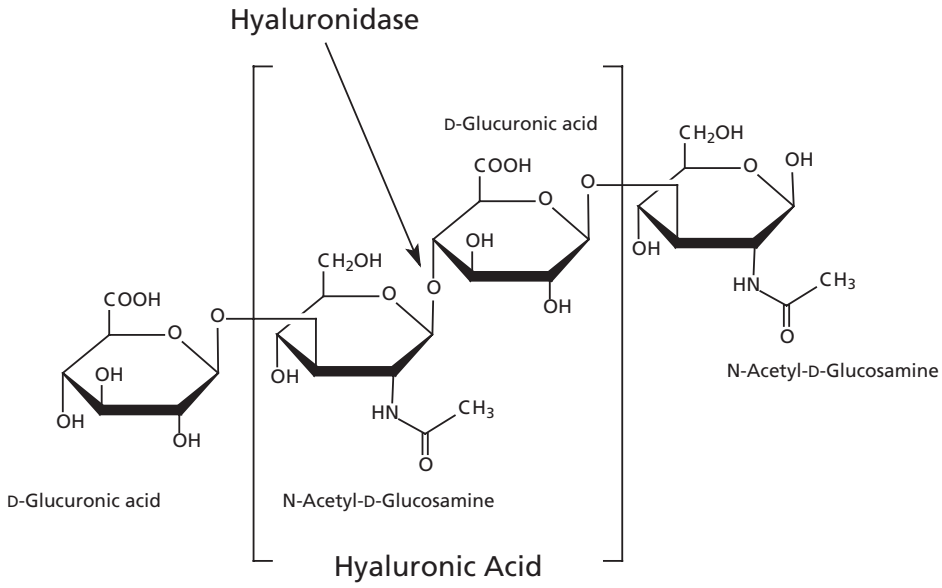
19. Bellemann, P., et al., *Anal. Biochem.*, **81**, 408 (1977)
20. Ives, H.E., et al., *J. Expt. Med.*, **148**, 1400 (1978)
21. Fain, J.N. and Loken, S.C., *J. Biol. Chem.*, **244**, 3500 (1969)
22. Berry, M.N., et al., Isolated Hepatocytes; Preparation, Properties and Applications. Elsevier. 1991

Hyaluronidase

Hyaluronidase is typically used as a supplement to proteases for tissue dissociation.[1] It catalyzes the random hydrolysis of 1,4-β-D-glycosidic linkages between N-acetyl-galactosamine or N-acetylgalactosamine sulfate and glucuronic acid in hyaluronic acid, chondroitin, chondroitin 4- and 6-sulfates, and dermatan.[2]

References

1. Hwang, W. S., et al., *Science*, **308**, 1777–1783 (2005)
2. Maija, c., et al., *PNAS*, **102**, 17834–17839 (2005)
3. http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/35.html



Composed of alternating residues of β-D-(1-3) glucuronic acid and β-D-(1-4)-N-acetylglucosamine

Hyaluronidase from sheep testes

Hyaluronate 4-glycanohydrolase; Hyaluronoglucosaminidase
[37326-33-3] E.C. 3.2.1.35; EC No. 2534643

These enzymes randomly cleave β-N-acetylhexosamine-[1→4] glycosidic bonds in hyaluronic acid, chondroitin, and chondroitin sulfates.

mol wt 55 kDa

One unit is based on the change in absorbance at 600 nm (change in turbidity) of a USP reference standard hyaluronidase which is assayed concurrently with each lot of this product.

References

1. Meyer, K., Hoffman, P., Linker, A., The *Enzymes* 2nd ed. (1960), **4**, 447

S: 22-24/25 EC No. 253-464-3

Type V, lyophilized powder, activity: ≥1,500 units/mg solid

-20°C

H6254-500MG	500 mg
H6254-1G	1 g

Type II, lyophilized powder, activity: ≥300 units/mg

Lyophilized powder containing lactose

-20°C

H2126-100MG	100 mg
H2126-500MG	500 mg
H2126-1G	1 g
H2126-5G	5 g

Type III, lyophilized powder, activity: ≥500 units/mg

Lyophilized powder containing 20–50% lactose

-20°C

H2251	

Hyaluronidase from *Streptomyces hyalurolyticus*

Hyaluronate Lyase from *Streptomyces hyalurolyticus*
[9001-54-1] E.C. 4.2.2.1; EC No. 2326141

lyophilized powder

This enzyme cleaves β-GlcNAc-[1→4] glycosidic bonds by elimination, yielding 4,5-unsaturated tetra- and hexasaccharides. Unlike other hyaluronidases, this enzyme is specific for hyaluronic acid and is inactive with chondroitin and chondroitin sulfate.[1]

Lit. cited: 1. Ohya, T., and Kaneko, Y., *Biochim. Biophys. Acta* **198**, 607 (1970)

EC No. 253-430-8 -20°C

H1136-1AMP	1 amp

Hyaluronidase from bovine testes

Hyaluronate 4-glycanohydrolase; Hyaluronoglucosaminidase
[37326-33-3] E.C. 3.2.1.35; EC No. 2534643

These enzymes randomly cleave β-N-acetylhexosamine-[1→4] glycosidic bonds in hyaluronic acid, chondroitin, and chondroitin sulfates.

mol wt ~55 kDa (four subunits of 14 kDa each)

S: 22-24/25 EC No. 253-464-3

Enzymes for Cell Detachment and Tissue Dissociation

Hyaluronidase cont'd

lyophilized powder, Type I-S, activity: 400–1000 units/mg solid

One unit is based on the change in absorbance at 600 nm (change in turbidity) of a USP reference standard hyaluronidase which is assayed concurrently with each lot of this product.

-20°C

H3506-100MG	100 mg
H3506-500MG	500 mg
H3506-1G	1 g
H3506-5G	5 g

essentially salt-free, lyophilized powder, Type IV-S, activity: 750–1500 units/mg solid

One unit is based on the change in absorbance at 600 nm (change in turbidity) of a USP reference standard hyaluronidase which is assayed concurrently with each lot of this product.

-20°C

H3884-50MG	50 mg
H3884-100MG	100 mg
H3884-500MG	500 mg
H3884-1G	1 g

Type IV-S, powder, mouse embryo tested

Recommended for dissolving cumulus mass in the isolation of mouse embryos.

aseptically processed

activity: 750–1500 units/mg solid

composition

Protein ~90% (biuret)

One unit is based on the change in absorbance at 600 nm (change in turbidity) of a USP reference standard hyaluronidase which is assayed concurrently with each lot of this product.

-20°C

H4272-30MG	30 mg

Type VIII, lyophilized powder, activity: ~300 units/mg

Prepared from sterile filtered solution of Type I-S.

One unit is based on the change in absorbance at 600 nm (change in turbidity) of a USP reference standard hyaluronidase which is assayed concurrently with each lot of this product.

-20°C

H3757-100MG	100 mg

Type VI-S, lyophilized powder, activity: 3,000–15,000 units/mg solid

Chromatographically purified

One unit is based on the change in absorbance at 600 nm (change in turbidity) of a USP reference standard hyaluronidase which is assayed concurrently with each lot of this product.

-20°C

H3631-3KU	3,000 units
H3631-15KU	15,000 units
H3631-30KU	30,000 units

DNase

DNAse is typically used to supplement proteases for tissue dissociation. DNase helps to reduce viscosity resulting from DNA released from damaged cells during harvesting.[1,2,3,4]

References

1. Davidson, D., et al., *J. Cystic Fibrosis,* **3**, 59-62 (2004)
2. West, C.M., et al., *J. Natl. Cancer Inst.*, **78**, 371-376 (1987)
3. Seglen, P.O., *Methods in Cell Biology,* **13**, 29 (1976)
4. Buitrago, A., rt al., *Biochem. Biophys. Res. Commun.,* **79**, 823 (1977)

Deoxyribonuclease I from bovine pancreas

Deoxyribonucleate 5′-oligonucleotido-hydrolase; DNase I
[9003-98-9] E.C. 3.1.21.1; EC No. 2326670

Used for the removal of DNA from protein samples.

mol wt ~31 kDa

One Kunitz unit will produce a ΔA_{260} of 0.001 per min per mL at pH 5.0 at 25 °C, using DNA, Type I or III as substrate. $[Mg^{2+}]$ = 4.2 mM.

References

1. Molloy, M.P., et al., Proteomic analysis of the *Escherichia coli* outer membrane. *Eur. J. Biochem.* **267**, 2871–2881 (2000)

Type IV, lyophilized powder, Protein: ~90%, activity: ≥2,000 Kunitz units/mg protein

Lyophilized powder containing calcium chloride

purified by chromatography

Derived from New Zealand-sourced pancreas

Protein determined by biuret.

protease ..... <0.05% chymotrypsin..... <0.5%

RNase ..... <0.02%

S: 22-24/25 EC No. 232-667-0 RTECS # RF0750000

-20°C

D5025-15KU	15,000 units
D5025-150KU	150,000 units
D5025-375KU	375,000 units
D5025-750KU	750,000 units

Type II, lyophilized powder, Protein: ~90%, activity: ≥2,000 units/mg protein

Contains calcium chloride

purified by chromatography

Derived from New Zealand-sourced pancreas

Protein determined by biuret.

chymotrypsin ..... <0.01% RNase..... <0.01%

protease ..... <0.005%

S: 22-24/25 EC No. 232-667-0 RTECS # RF0750000 -20°C

D4527-10KU	10,000 units
D4527-20KU	20,000 units
D4527-40KU	40,000 units
D4527-200KU	200,000 units
D4527-500KU	500,000 units

Type II-S, lyophilized powder, Protein: ~90%, activity: ≥2,000 units/mg protein

Lyophilized powder containing calcium chloride

purified by chromatography

sterile-filtered

vial of ~11 mg protein

Derived from New Zealand-sourced pancreas

Protein determined by biuret.

endotoxin .....tested RNase..... <0.01%

chymotrypsin .....≤0.01% protease ..... <0.005%

S: 22-24/25 EC No. 232-667-0 RTECS # RF0750000 -20°C

D4513-1VL	1 vial

DNase cont'd

▸ lyophilized powder, Protein: ≥85%, activity: 400–800 Kunitz units/mg protein

Crude preparation, contains calcium chloride

Derived from New Zealand-sourced pancreas

Protein determined by biuret.

RNase ..... <0.02%

S: 22-24/25 EC No. 232-667-0 RTECS # RF0750000 -20°C

DN25-10MG	10 mg
DN25-100MG	100 mg
DN25-1G	1 g
DN25-5G	5 g

▸ lyophilized powder, Protein: ~80%, activity: ≥1,500 Kunitz units/mg protein

Contains glycine

purified by chromatography

Protein determined by biuret.

RNase ..... <0.01%

S: 22-24/25 EC No. 232-667-0 RTECS # RF0750000 -20°C

DNEP-5MG	5 mg

▸ from bovine, recombinant, expressed in proprietary host

Supplied as a solution in 20 mM HEPES, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 1 mM DTT, 50% glycerol, pH 7.5.

This product is prepared essentially free of RNase and protease activity.

S: 23-24/25

-20°C WET ICE

D7691-5UG	5 µg
D7691-25UG	25 µg

Elastase

Pancreatic elastase has the unique ability to digest native elastin. For this reason, it is often used to supplement other proteases for the dissociation of tissues containing higher amounts of elastin connective fibers, such as lung tissue.[1,2] It is a serine protease with preferential cleavage for the carboxyl-side of alanine.[3]

References

1. Phillips, H.J., *Invitro*, **8**, 101–105 (1972)
2. Ives, H.E., et al., J. *Expt. Med.*, **148**, 1400 (1978)
3. http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/4/21/36.html

Elastase, pancreatic from porcine pancreas

Elastase from hog pancreas; Pancreatopeptidase E
[39445-21-1] E.C. 3.4.21.36; EC No. 2544536

One unit will hydrolyze 1.0 µmole of N-succinyl-L-Ala-Ala-Ala-*p*-nitroanilide per min, pH 8.0 at 25 °C.

EC No. 254-453-6

▸ lyophilized powder, activity: 3–6 units/mg protein (biuret), cell culture tested

solubility

H_2O ..... soluble

trypsin ..... <50 BAEE units/mg protein

R: 36/37/38-42 S: 22-24-26-36/37 -20°C

E7885-1MG	1 mg
E7885-5MG	5 mg
E7885-20MG	20 mg

▸ Type IV, Protein: ~70%, lyophilized powder, activity: ≥4 units/mg protein (biuret)

Contains sodium carbonate.

A further purification of Type III, E0127, by affinity chromatography to reduce trypsin activity

trypsin ..... <50 BAEE units/mg protein

R: 36/37/38-42 S: 22-24-26-36/37 -20°C

E0258-1MG	1 mg
E0258-5MG	5 mg
E0258-10MG	10 mg
E0258-20MG	20 mg
E0258-50MG	50 mg

▸ Type III, lyophilized powder, Protein: ~70%, activity: ≥4 units/mg protein

Contains trypsin activity

Contains sodium carbonate.

2x crystallized and chromatographically purified

R: 36/37/38-42 S: 22-24-26-36/37 -20°C

E0127-5MG	5 mg
E0127-10MG	10 mg
E0127-20MG	20 mg
E0127-100MG	100 mg

▸ Type II-A, lyophilized powder, Protein: ~70%, activity: ≥1 units/mg protein (biuret)

Contains sodium carbonate.

2x Crystallized

trypsin .....≤500 BAEE units/mg protein

Ref: 1. Baumstark, J.S., et al., *Biochim. Biophys. Acta* **77**, 676 (1963)

R: 36/37/38-42 S: 22-24-26-36/37 -20°C

E6883-10MG	10 mg
E6883-25MG	25 mg
E6883-50MG	50 mg
E6883-100MG	100 mg
E6883-250MG	250 mg

▸ Type I, aqueous suspension, activity: ≥4 units/mg protein

contains ~0.01% thymol

2x crystallized

Package size based on protein content

trypsin .....≤50 BAEE units/mg protein

References

1. Baumstark, J.S., et al., *Biochim. Biophys. Acta* **77**, 676 (1963)

R: 36/37/38-42 S: 23-24-26-36/37 2-8°C

E1250-10MG	10 mg
E1250-25MG	25 mg
E1250-50MG	50 mg
E1250-100MG	100 mg
E1250-500MG	500 mg

Enzymes for Cell Detachment and Tissue Dissociation

Papain

Papain is a relatively nonspecific sulfhydryl protease derived from papaya latex. Papain is used alone or in addtion to other proteases such as collagenase.[1,2,3,4] In some instances, the crude papaya latex preparation has been found to be most efficient for cell dissociation.[5]

References

1. He, W., *J. Neurosci*, **21**, 8854-8862 (2001)
2. Guidry C., *Invest. Ophthalmol. Vis. Sci.*,**37**, 740-52 (1996)
3. Kobayashi, S., *J Pharmacol. Toxicol. Methods*, **45**, 199–205 (2001)
4. Piper, A.S. and Large, W.A., *J. Physiol.*, **555**, 397–408 (2003)
5. Customer communications

Papain from papaya latex

Papainase
[9001-73-4] E.C. 3.4.22.2

A cysteine protease that cleaves peptide bonds of basic amino acids, leucine, or glycine. pH optimum 6.0–7.0

Also hydrolyzes esters and amides.

Used to produce Fab fragments of antibodies.[1] Also used for cell dissociation since it has been shown to be more effective and less damaging with certain tissues.[2,3]

mol wt 21 kDa

One unit will hydrolyze 1.0 µmole of BAEE per min at pH 6.2 at 25°C.

Lit. cited:

1.Y. Ozari, J. Jagur-Grodzinski, *J. Chem. Soc. Chem. Commun.* 295 (1974)

References

2. M.A. Andrews, *Carbohydr. Res.* **194**, 1 (1989)
3. H. Cai et al., *Anal. Chem.* **70**, 580 (1998)
4. Dreyfus, Cheryl F., Black, Ira B., and, P. Michael Conn, ed., *Methods in Neuroscience*, Academic Press, Inc (San Diego: 1990), **2**, 10
5. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY: 1988), 626–628
6. Townes-Anderson, E., et al., Rod Cells Dissociated from Mature Salamander Retina: Ultrastructure and Uptake of Horseradish Peroxidase. *J. Cell Biol.* **100**, 175 (1985)

lyophilized powder, activity: ≥10 units/mg protein ($E^{1\%}_{280}$)

Lyophilized powder containing sodium chloride and sodium acetate 2× Crystallized

References

1. Fruton, J.S., *Adv. Enzymol.* **53**, 239 (1982)
2. Glazer, A.N., Smith, E.L., *The Enzymes* (1971), **3**, 501
3. Arnon, R., Meth. *Enzymol.* **19**, 226 (1970)

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-627-2 Light sensitive
RTECS # RU4950000 –20°C

P4762-25MG	25 mg
P4762-50MG	50 mg
P4762-100MG	100 mg
P4762-500MG	500 mg
P4762-1G	1 g
P4762-5G	5 g

crude powder, activity: 1.5–10 units/mg solid

Not standardized with lactose or other adulterants.

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-627-2 Light sensitive
RTECS # RU4950000 2–8°C

P3375-25G	25 g
P3375-100G	100 g
P3375-1KG	1 kg

lyophilized powder, aseptically filled

Prepared from P4762

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-627-2 Light sensitive
RTECS # RU4950000 –20°C

P5306-25MG	25 mg

lyophilized powder, activity: 0.5–2 units/mg solid

Useful in studies of the natural activities of the papaya latex.

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-627-2 Light sensitive
RTECS # RU4950000 2–8°C

P3250-25G	25 g
P3250-100G	100 g
P3250-1KG	1 kg

buffered aqueous suspension, 2× Crystallized, activity: 16–40 units/mg protein

Suspension in 0.05 M sodium acetate, pH 4.5, containing 0.01% thymol

R: 42/43 S: 36 2–8°C

P3125-25MG	25 mg
P3125-100MG	100 mg
P3125-250MG	250 mg
P3125-500MG	500 mg
P3125-1G	1 g

Protease Type XIV

Pronase is an extremely nonspecific protease that has found applications the dissociation of various tissues.[1,2]

References

1. Xie, J. and Haslam, S., *Endocrinology,* **138**, 2466–2473 (1997)
2. Vanoye , C., et al., *Am. J. Physiol. Cell Physiol.*, **276**, C279–C284 (1999)

Protease from *Streptomyces griseus*

Actinase E; Pronase E
[9036-06-0] EC No. 2329666

Pronase is also used in nucleic acid isolation procedures in incubations of 0.5–3.0 hours supplemented with 0.2% sodium dodecyl sulfate and 10 mM EDTA.

Features & Benefits

- highly stable in pH range of 5.0 to 9.0, with peak activity at pH 8.8
- compatible with many DNA and RNA isolation buffers
- broad substrate specificity

A mixture of at least three proteolytic activities including an extracellular serine protease. In general, serine proteases display a wide range of substrate specificities, which are believed to be mediated by an active site composed of one Asp, one His, and a Ser residue in the molecule. This enzyme prefers to hydrolyze peptide bonds on the carboxyl side of glutamic or aspartic acid.

Collected from culture broth of *S. griseus* and purified by successive column procedures.

Type XIV, activity: ≥3.5 units/mg solid, powder

Contains calcium acetate.

One unit will hydrolyze casein to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

S: 22-24/25 EC No. 232-909-5 RTECS # UK9595000

–20°C

P5147-100MG	100 mg
P5147-1G	1 g
P5147-5G	5 g

Trypsin

Trypsin is a serine protease commonly used for detachment of adherent cell lines and dissociation of tissues. Crude trypsin preparations have typically been found to be more efficient for both applications.

Cultured cells are most commonly removed from the culture substrate by treatment with trypsin, or trypsin-EDTA solutions. The concentration of trypsin can range from 0.025% to 0.5%. Incubating cells with too high a trypsin concentration for too long a time period will damage cell membranes and kill the cells.

For the dissociation of tissues, trypsin has been used alone[1] or as a supplement to other enzymes.[2,3]

Sigma also offers cGMP grade trypsins. Inquire with your local sales representative.

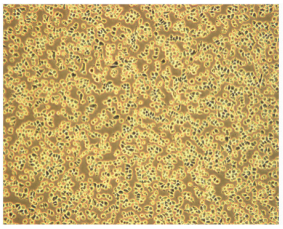
References

1. Rheinwald, J.G., and Green, H., *Cell*, **6**, 331–344 (1975)
2. Stingl, J., et al.,Meth. *Mo. Biol.*, **290**, 249–263 (2005)
3. Tong, W., *Meth. Enzymol*,. **32**, 745 (1974)

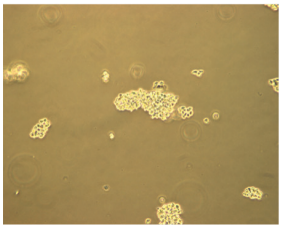
Trypzean™

Trypzean™ is manufactured by Sigma utilizing ProdiGene's proprietary transgenic plant protein expression system. Trypzean eliminates the introduction of animal source contaminants found in traditional bovine and porcine trypsins.

Native Trypsin 1 × Solution

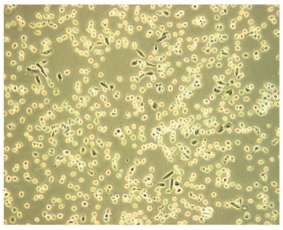


5 minutes

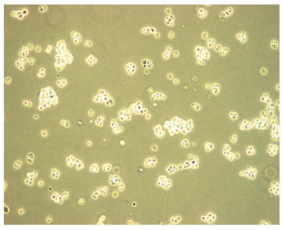


10 minutes

TrypZean 1 × Solution



5 minutes



10 minutes

The above pictures show Vero cells (grown in DME/F12 supplemented with 10% FBS) at various timed exposures to native trypsin and TrypZean.

TrypZean™

Trypsin bovine
[9002-07-7] E.C. 3.4.21.4

For dissociation of cultured cells.

recombinant, expressed in corn, lyophilized powder, activity: ≥3650 units/mg solid (USP)

Eliminates the introduction of animal source contaminants found in traditional bovine and porcine trypsins.

R: 36/37/38-42/43 S: 23-24-26-36/37 Moisture sensitive –20°C

T3568-10MG	10 mg
T3568-100MG	100 mg

TrypZean™ Solution, 1X

Trypsin

R: 36/37/38-42 S: 23-24-26-36/37

recombinant, expressed in corn, sterile-filtered

Eliminates the introduction of animal source contaminants found in traditional bovine and porcine trypsins.

aqueous solution

–20°C

T3449-100ML	100 mL
T3449-500ML	500 mL

Enzymes for Cell Detachment and Tissue Dissociation

Trypsin Powders

Trypsin from bovine pancreas

[9002-07-7] E.C. 3.4.21.4; EC No. 2326508

mol wt 23.8 kDa

One BTEE unit = 320 ATEE units

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-650-8 RTECS # YN5075000

essentially salt-free, lyophilized powder, activity: ≥10,000 BAEE units/mg protein

Derived from New Zealand-sourced pancreas

TPCK treated; the treatment with L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK) reduces the chymotrypsin activity which is usually present in trypsin.

Dialyzed

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

chymotrypsin ..... ≤0.1 BTEE units/mg protein

Moisture sensitive -20°C

T1426-50MG	50 mg
T1426-100MG	100 mg
T1426-250MG	250 mg
T1426-500MG	500 mg
T1426-1G	1 g
T1426-5G	5 g
T1426-100G	100 g

Type I, activity: ~10,000 BAEE units/mg protein

Features & Benefits

Yields a denser product than a lyophilized powder. Derived from New Zealand-sourced pancreas

Ethanol precipitate

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

chymotrypsin ..... <4 BTEE units/mg protein

Moisture sensitive -20°C

T8003-100MG	100 mg
T8003-500MG	500 mg
T8003-1G	1 g
T8003-10G	10 g

lyophilized powder, activity: ≥7,500 BAEE units/mg solid

Lyophilized powder containing lactose

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

chymotrypsin ..... <3 BTEE units/mg solid

Moisture sensitive -20°C

T4665-100MG	100 mg
T4665-500MG	500 mg
T4665-1G	1 g
T4665-5G	5 g
T4665-10G	10 g

Type XI, lyophilized powder, activity: ≥6,000 BAEE units/mg protein

Derived from New Zealand-sourced pancreas

DPCC treated (diphenylcarbamyl chloride) to reduce the chymotrypsin activity which is usually present in trypsin.

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

salt ..... essentially free

chymotrypsin ..... <0.1 BTEE units/mg protein

Moisture sensitive -20°C

T1005-250MG	250 mg
T1005-500MG	500 mg
T1005-1G	1 g
T1005-5G	5 g

activity: ≥2,500 USP units/mg solid, meets USP testing specifications

purified by crystallization

Derived from New Zealand-sourced pancreas

solubility

H_2O ..... soluble

saline ..... soluble

Hygroscopic -20°C

T7309-1G	1 g
T7309-10G	0 g

essentially salt-free, lyophilized powder, activity: 9,000–13,000 BAEE units/mg protein, cell culture tested

Contains chymotrypsin activity.

Derived from New Zealand-sourced pancreas

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

Moisture sensitive -20°C

T9935-50MG	50 mg
T9935-100MG	100 mg

essentially salt-free, lyophilized powder, activity: 10,000–15,000 BAEE units/mg protein

aseptically filled

Derived from New Zealand-sourced pancreas

TPCK treated

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

Protein determined by ($E^{1\%}_{280}$)

chymotrypsin ..... <0.1 BTEE units/mg protein

Moisture sensitive -20°C

T8802-50MG	50 mg
T8802-100MG	100 mg

Trypsin from porcine pancreas

[9002-07-7] E.C. 3.4.21.4; EC No. 2326508

mol wt 23.8 kDa

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

One BTEE unit = 320 ATEE units

Type IX-S, lyophilized powder, activity: 13,000–20,000 BAEE units/mg protein

chymotrypsin .....≤1 units/mg protein

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-650-8
Moisture sensitive RTECS # YN5075000 -20°C

T0303-1G	1 g
T0303-10G	10 g

lyophilized powder, activity: 1,000–1,500 BAEE units/mg solid, cell culture tested

Contains chymotrypsin and elastase activities.

Lyophilized powder containing lactose

porcine parvovirus.....none detected (9 CFR)

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-650-8
Moisture sensitive RTECS # YN5075000 -20°C DRY ICE

T4799-5G	5 g
T4799-10X5G	10 × 5 g
T4799-10G	10 g
T4799-25G	25 g
T4799-100G	100 g
T4799-500G	500 g

lyophilized powder, Type II-S, activity: 1,000–2,000 BAEE units/mg solid

Lyophilized powder containing lactose

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-650-8
Moisture sensitive RTECS # YN5075000 -20°C

T7409-1G	1 g
T7409-10G	10 g
T7409-100G	100 g

activity: 1,000–1,500 BAEE units/mg solid, γ-irradiated, cell culture tested

Contains chymotrypsin and elastase activities.

Lyophilized powder containing lactose

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-650-8
Moisture sensitive RTECS # YN5075000 2-8°C

T5266-500MG	500 mg

tablet, 1 mg tablet

For use in immunohistochemical procedures to enhance staining and to unmask antigens after routine fixation and processing.

Dissolve 1 tablet in 1 mL deionized water to yield a ready-to-use buffered solution of 1 mg/mL trypsin containing 4 mM $CaCl_2$, 200 mM Tris, pH 7.7 at 25 °C.

R: 36/37/38-42/43 S: 22-24-26-36/37 -20°C

T7168-20TAB	20 tablets
T7168-50TAB	50 tablets

Trypsin Solutions

Trypsin solution from porcine pancreas

[9002-07-7]

1×, 2.5 g porcine trypsin per liter in Hanks' Balanced Salt Solution with phenol red, sterile-filtered, cell culture tested

Porcine parvovirus.....none detected (9 CFR)

◆ -20°C DRY ICE

T4424-100ML	100 mL
T4424-500ML	500 mL

Trypsin solution from porcine pancreas

[9002-07-7]

Porcine parvovirus..... none detected (9 CFR)

R: 36/37/38-42 S: 23-45 EC No. 232-650-8 RTECS # YN5075000 ◆

10×, 25 g porcine trypsin per liter in 0.9% sodium chloride, sterile-filtered, cell culture tested

-20°C DRY ICE

T4549-20ML	20 mL
T4549-100ML	100 mL

10×, 25 g porcine trypsin per liter in Hanks' Balanced Salt Solution with phenol red, sterile-filtered, cell culture tested

-20°C DRY ICE

T4674-100ML	100 mL

Trypsin-EDTA solution

1×, 0.5 g porcine trypsin and 0.2 g EDTA • 4Na per liter of Hanks' Balanced Salt Solution with phenol red, sterile-filtered, cell culture tested

Porcine parvovirus.....none detected (9 CFR)

◆ -20°C DRY ICE

T3924-100ML	100 mL
T3924-500ML	500 mL

Trypsin-EDTA solution

Porcine parvovirus..... none detected (9 CFR)

◆

0.25%, 2.5 g porcine trypsin and 0.2 g EDTA • 4Na per liter of Hanks' Balanced Salt Solution with phenol red, sterile-filtered, cell culture tested

-20°C DRY ICE

T4049-100ML	100 mL
T4049-500ML	500 mL

1×, 500 BAEE units porcine trypsin and 180 µg EDTA • 4Na per ml in Dulbecco's Phosphate Buffered Saline without calcium and magnesium, sterile-filtered, cell culture tested

For use with endothelial cell cultures.

-20°C DRY ICE

T4299-100ML	100 mL

Trypsin-EDTA solution

10×, 5.0 g porcine trypsin and 2 g EDTA • 4Na per liter of 0.9% sodium chloride, sterile-filtered, cell culture tested

Porcine parvovirus.....none detected (9 CFR)

◆ -20°C DRY ICE

T4174-20ML	20 mL
T4174-100ML	100 mL

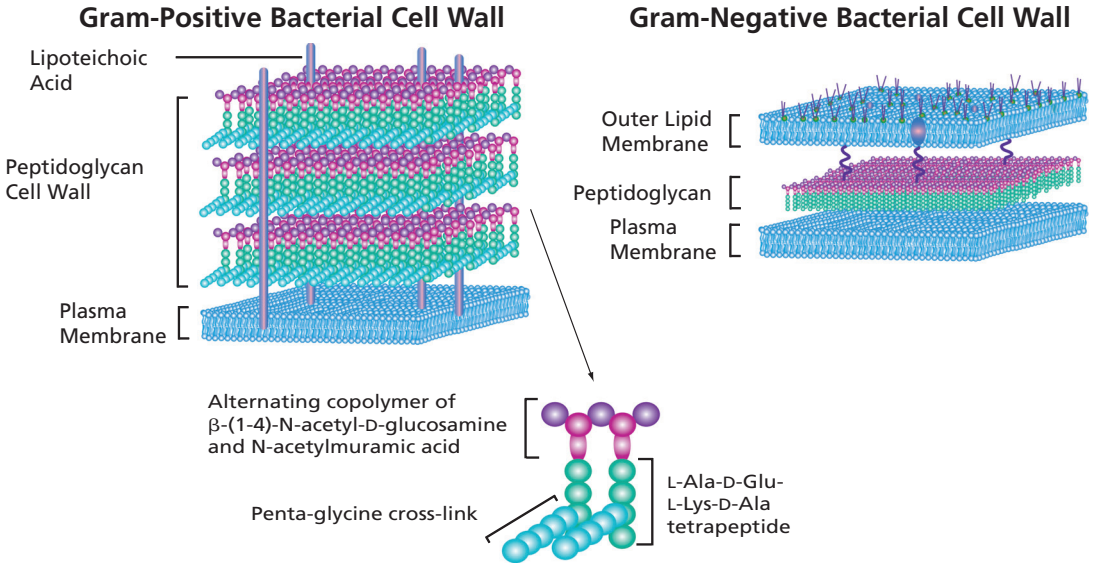
Enzymes for Cell Lysis and Protoplast Preparation

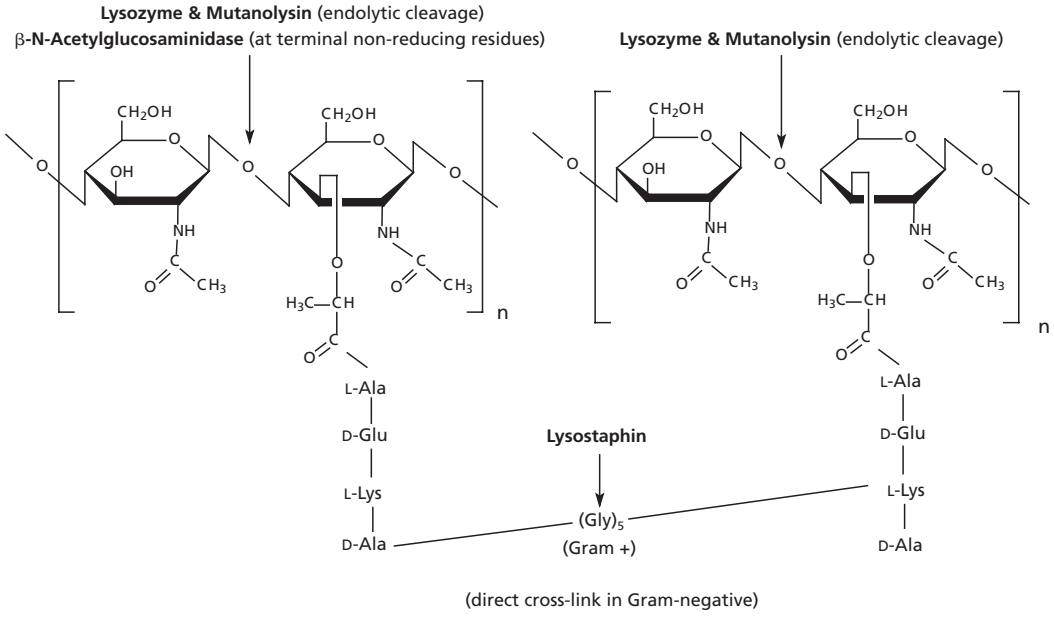
Enzymatic lysis and protoplast preparation is very specific to cell wall and membrane morphology. Each cell type requires optimization of the type and concentration of enzymes used, as well as other components, such as detergents, used in the digestion buffer. Sigma offers several preformulated kits for cell lysis and extraction and purification of DNA, RNA and proteins. For more information visit our Web site at: sigma-aldrich.com/dnapurification, sigma-aldrich.com/lysis

Bacterial Cell Lysis and Protoplast Preparation

The cell wall of Gram-positive bacteria is composed of multiple layers of peptidoglycan which comprises approx 90% of the cell wall structure. Peptidoglycan is a polymer of β-(1-4)-N-Acetyl-D-glucosamine units. Alternating residues are modified to form N-acetylmuramic acid with the addition of lactate to form branching links to a tetrapeptide. The tetrapeptides of adjacent polymers are linked by pentaglycine bridges. The cross-linked peptidoglycan polymers form a mesh-like network over a phospholipid bilayer plasma membrane.

The Gram-negative cell wall is composed of an outer lipid bilayer, which, in addition to phospholipids, is also covered with lipopolysaccharide moieties. Lipoproteins link the outer lipid membrane to the thin peptidoglycan layer in the periplasmic space. The inner plasma membrane is a phospholipid bylayer.





Achromopeptidase from *Achromobacter lyticus*

[123175-82-6] E.C. 3.4.21.50

Achromopeptidase is a lysyl endopeptidase with a MW of ~27 kDa. It is useful for lysis of Gram-positive bacteria that are resistant to lysozyme.

pH Optimum for activity: pH 8.5–9

Approximately 500–1,500 un/mL achromopeptidase can be used to lyse cells at a density of OD_{600}=0.6 over 2 hours at 37 °C.

One unit will produce a change in A_{600} of 0.01 per minute per mL at pH 8.0 at 37 °C using a suspension of *Micrococcus lysodeikticus* as substrate (1 cm light path).

collagenase.....present

References

1. Ezaki, T and Suzuki, S., J. Clin. Microbiol. **16**, 844–846 (1982)

R: 42/43 S: 36

▸ partially purified powder, activity: 20,000–40,000 units/mg solid

Partially purified powder containing lactose.

-20°C

A3422-25KU	25,000 units
A3422-50KU	50,000 units

▸ lyophilized powder, activity: 800–3,200 units/mg solid

Crude powder containing lactose

-20°C

A3547-100KU	100,000 units
A3547-500KU	500,000 units
A3547-1MU	1,000,000 units

▸ lyophilized powder, Protein: ~5%, activity: 300–600 units/mg solid

Crude powder containing salts and medium components

-20°C

A7550-100KU	100,000 units

Labiase from *Streptomyces fulvissimus* TU-6

Labiase from *Streptomyces fulvissimus* is an enzyme preparation useful for the lysis of many Gram-positive bacteria such as *Lactobacillus, Aerococcus,* and *Streptococcus.*

Labiase contains β-N-acetyl-D-glucosaminidase and lysozyme activity.

pH Optimum for activity: pH ~4

pH Optimum for stability: pH 4-8

References:

1. Niwa, T. et al., *J. Microbiol. Methods* **61**, 251–260 (2005)

2-8°C

L1414-500MG	500 mg

Lysostaphin from *Staphylococcus staphylolyticus*

Glycyl-glycine Endopeptidase
[9011-93-2] E.C. 3.4.24.75

Lysostaphin is a zinc endopeptidase with a molecular weight of approximately 25 kDa. Because lysostaphin cleaves the polyglycine cross-links in the peptidoglycan layer of the cell wall of *Staphylococcus* species it has been found useful for cell lysis and also as a potential antimicrobial therapeutic.

pH Optimum for activity: ~7.5

References

1. Boneca, et al., Characterization of Staphylococcus aureus cell wall glycan strands, evidence for a new β-N-acetyl glucosaminidase activity. *J. Biol. Chem.* **275**, 9910–9918 (2000)
2. Trayer, H. R., and Buckley, C. E., *J. Biol. Chem.* **245**, 4842–4846 (1970)
3. Browder, H.P., et al., *Biochem. Biophys. Res. Commun.* **19**, 383 (1965)

S: 22-24/25 RTECS # OL5985000 -20°C

▸ lyophilized powder, Protein: ~60%, activity: ≥500 units/mg protein

Package size based on protein content

One unit will reduce the turbidity (A_{620}) of a suspension of *Staphylococcus aureus* cells from 0.250 to 0.125 in 10 min at pH 7.5 at 37 °C in a 6.0 mL reaction mixture.

-20°C

L7386-1MG	1 mg
L7386-5MG	5 mg
L7386-15MG	5 mg

▸ lyophilized powder, >97% (SDS-PAGE), Protein: ~50%, activity: ≥2,000 units/mg protein

Package size based on protein content

Contains potassium phosphate buffer salts and sodium chloride

Affinity purified

One unit will reduce the turbidity (A_{620}) of a suspension of *Staphylococcus aureus* cells from 0.250 to 0.125 in 10 min at pH 7.5 at 37 °C in a 6.0 mL reaction mixture.

-20°C

L4402-.5MG	0.5 mg
L4402-2MG	2 mg
L4402-5MG	5 mg

▸ aseptically filled

Prepared from L7386

One unit will reduce the turbidity (A_{620}) of a suspension of *Staphylococcus aureus* cells from 0.250 to 0.125 in 10 min at pH 7.5 at 37 °C in a 6.0 mL reaction mixture.

-20°C

L2898-1MG	1 mg

Lysozyme

Mucopeptide N-acetylmuramoylhydrolase; Muramidase

E.C. 3.2.1.17

Lysozyme from chicken egg white

[12650-88-3] EC No. 2326204

Lysozyme hydrolyzes β(1→4) linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrin. Gram-positive cells are quite susceptible to this hydrolysis as their cell walls have a high proportion of peptidoglycan. Gram-negative bacteria are less susceptible due to the presence of an outer membrane and a lower proportion of peptidoglycan. However, these cells may be hydrolyzed in the presence of EDTA that chelates metal ions in the outer bacterial membrane.

The enzyme is active over a broad pH range (6.0 to 9.0). At pH 6.2, maximal activity is observed over a wider range of ionic strengths (0.02 to 0.100 M) than at pH 9.2 (0.01 to 0.06 M).

single-chain mol wt 14.7 kDa

References

1. Jolles, P., *Angew. Chem. Int. Ed. Engl.* **8**, 227–239 (1969)
2. Rupley, J.A., *Biochim. Biophys. Acta* **83**, 245–255 (1964)
3. Holler, H., et al., *Biochemistry* **14**, 2377–2385 (1975)
4. Canfield, R.E., *J. Biol. Chem.* **238**, 2698–2707 (1963)
5. Davies, R.C., et al., *Biochim. Biophys.* Acta **178**, 294–305 (1969)

S:22-24/25 EC No. 235-747-3 RTECS # OL5989000

Enzymes for Cell Lysis and Protoplast Preparation

Lysozyme cont'd

lyophilized powder, Protein: ~95%, activity: ~50,000 units/mg protein ($E^{1\%}_{282}$)

Dialyzed and lyophilized, containing buffer salts as sodium acetate and sodium chloride

3× crystallized

One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL reaction mixture (1 cm light path).

-20°C

L6876-1G	1 g
L6876-5G	5 g
L6876-10G	10 g
L6876-25G	25 g
L6876-100G	100 g

lyophilized powder, Protein: ~95%, activity: ~50,000 units/mg protein ($E^{1\%}_{282}$)

Lysozyme hydrolyzes the β-1,4 linkages between N-acetylmuramic acid and N-acetylglucosamine, a polysaccharide backbone of peptidoglycans in the cell wall structure of many microorganisms. This is particularly useful for lysing Gram-positive and Gram-negative bacteria for subsequent nucleic acid extraction.

Features & Benefits

- Highly purified by repeated crystallization and dialysis
- Each lot is use-tested for isolation of plasmid DNA from *E. coli*

Lyophilized powder, essentially salt-free

3× crystallized

One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25° C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL reaction mixture (1 cm light path).

Lit. cited:

1. Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual* 2nd ed., Cold Spring Harbor Laboratory (Plainview, NY: 1989), 1.29

References

2. Jolles, P., Lysozymes: a chapter of molecular biology. *Angew. Chem. Int. Ed. Engl.* **4**, N227–239 (1969)

-20°C

L7651-1G	1 g
L7651-5G	5 g
L7651-10G	10 g
L7651-25G	25 g
L7651-100G	100 g

lyophilized powder, activity: ~50,000 units/mg protein ($E^{1\%}_{282}$), Protein: ~85%

Lyophilized powder containing sodium acetate buffer salts and sodium chloride

Grade III

3× Crystallized

One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL reaction mixture (1 cm light path).

-20°C

L7001-1G	1 g
L7001-5G	5 g
L7001-10G	10 g
L7001-25G	25 g

aseptically filled

Prepared from L6876

One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25 °C using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL reaction mixture (1 cm light path).

-20°C

L7773-50MG	50 mg

Lysozyme from human milk

[12671-19-1]

lyophilized powder, activity: ≥100,000 units/mg protein

Lyophilized powder containing sodium phosphate and sodium chloride

composition

Protein ~10% ($E^{1\%}_{280}$)

One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL reaction mixture (1 cm light path).

-20°C

L6394-25KU	25,000 units
L6394-100KU	100,000 units

recombinant, expressed in rice, activity: ≥100,000 units/mg protein

≥90% (SDS-PAGE)

One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL reaction mixture (1 cm light path).

S: 22-24/25 -70°C DRY ICE

L1667-10MG	10 mg
L1667-100MG	100 mg

Lysozyme from human neutrophils

[9001-63-2]

≥95% (SDS-PAGE), lyophilized powder, activity: ≥100,000 units/mg protein ($E^{1\%}_{280}$)

Lyophilized from 50 mM sodium acetate, pH 6.0, with 100 mM NaCl

One unit will produce a ΔA_{450} of 0.001 per min at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL

reaction mixture (1 cm light path).

References

1. Shugar, D., *Biochim. Biophys.* Acta **8**, 302 (1952)

-20°C

L8402-.1MG	0.1 mg

Mutanolysis

Mutanolysis from *Streptomyces globisporus* ATCC 2553

[55466-22-3]

Mutanolysis is an N-acetylmuramidase. Like lysozyme, it is a muralytic enzyme that cleaves the β-N-acetylmuramyl-(1→4)-N-acetylglucosamine linkage of the bacterial cell wall polymer peptidoglycan-polysaccharide. Its carboxy terminal moieties are involved in the recognition and binding of unique cell wall polymers. Mutanolysin lyses *Listeria* and other Gram-positive bacteria such as *Lactobacillus* and *Lactococcus.*

Provides gentle cell lysis for the isolation of easily degradable bio-molecules and RNA from bacteria. It has been used in the formation of spheroplasts for isolation of DNA.

mol wt 23 kDa

One unit will produce a ΔA_{600} of 0.01 per minute at pH 6.0 at 37 °C in a 1 mL volume using a suspension of *Streptococcus faecalis* cell wall as substrate.

S: 22-24/25

Mutanolysin cont'd

activity: ≥4,000 units/mg protein (biuret), Chromatographically purified

Lyophilized powder containing Ficoll® and sodium succinate buffer salts

–20°C

M9901-1KU	1,000 units
M9901-5KU	5,000 units
M9901-10KU	10,000 units
M9901-50KU	50,000 units

aseptically filled, lyophilized powder, activity: ≥4,000 units/mg protein (biuret)

Lyophilized powder containing Ficoll and sodium succinate buffer salts

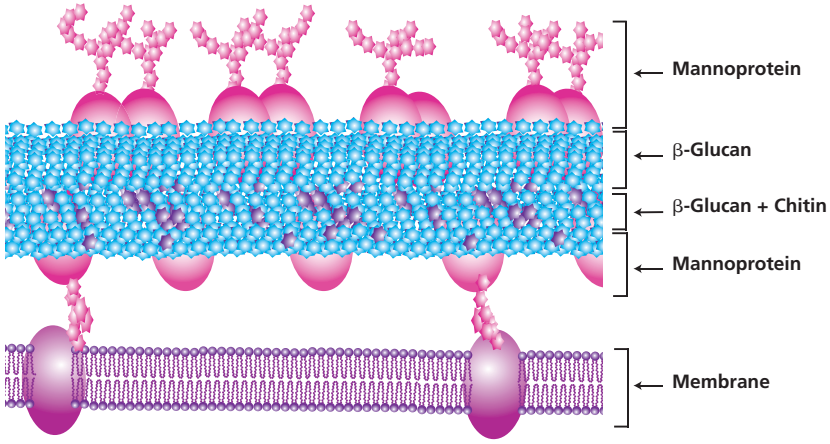
Prepared from M9901

–20°C

M4782-5KU	5,000 units

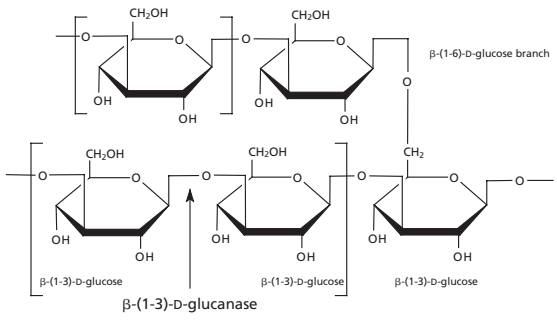
Yeast Cell Lysis and Protoplast Preparation

Yeast Cell Wall



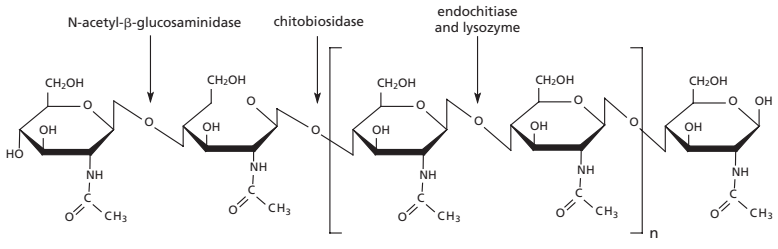
In Yeast, the cell wall comprises ~30 % of the dry weight of the cell. The yeast cell wall is made of ~25% helical β-(1-3) and β-(1-6)-d-glucans and ~25% oligomannans, ~20 % protein, ~10% lipids, and some chitin. The protein component exists predominantly as a mannoprotein complex. Covalent linkages are reported to exist as β-(1-4)-linkages between the reducing ends of chitin and the nonreducing end of β-(1-3)-glucans[1] as well as among glycoproteins, β-(1-6)-glucans, and β-(1-3)-glucans.[2]

Yeast β-Glucan



Polymer of β-(1-3)-D-glucopyranosyl units with branching at β-(1-6)-D-glycopyranosyl units

Chitin



Polymer of β-(1-4)-N-Acetyl-D-glucosamine units

References

1. Kollár, R., et al., E. *J. Biol. Chem,*. **270**, 1170–1178 (1995)
2. Kapteyn, J. C., et al., *Glycobiology,* **6**, 337–345 (1996)

Enzymes for Cell Lysis and Protoplast Preparation

Lysing Enzymes

from *Rhizoctonia solani*

Kitalase

crude powder

Main enzymatic acitivity is β–(1-3)-glucanase, also reported to contain protease, pectinase, and amylase activities.

References

1. Tsuchiya, D., and Taga, M., *Phytopathology* **91**, 354–360 (2001)

2-8°C

L8757-1G	1 g

from *Trichoderma harzianum*

Glucanex®

lyophilized powder

Contains β-glucanase, cellulase, protease, and chitinase activities

Used for yeast spheroplast transformation by hydrolyzing poly(1-3) glucose of the yeast cell wall glucan. Also used to retrieve DNA plugs from agarose gels.

References

1. Petit, J., et al., Glucanex: a cost-effective yeast lytic enzyme. *Trends Genet.* **10**, 4 (1994)
2. De Sampaio, G., et al., A constitutive role for GPI anchors in Saccharomyces cerevisiae: cell wall targeting. *Mol. Microbiol.* **34**, 247–256 (1999)

A product of Novozyme Corp.

2-8°C

L1412-5G	5 g
L1412-10G	10 g
L1412-25G	25 g

Lyticase from *Arthrobacter luteus*

[37340-57-1]

Lyticase hydrolyzes poly-β-(1-3)-glucose such as yeast cell wall glucan. Yeast cells are difficult to disrupt because the cell walls may form capsules or resistant spores. DNA can be extracted from yeast by using lysing enzymes such as lyticase, chitinase, zymolase, and gluculase to induce partial spheroplast formation; spheroplasts are subsequently lysed to release DNA. Lyticase is preferred to digest cell walls of yeast and generate spheroplasts from fungi for transformation.

Reported to be useful for lysis of *Ashbya, Candida, Debaryomyces, Eremothecium, Endomyces, Hansenula, Hanseniaspora, Kloeckera, Kluyveromyces, Lipomyces, Metschikowia, Pichia, Pullularia, Torulopsis, Saccharomyces, Saccharomycopsis, Saccharomycodes,* and *Schwanniomyces* species.

One unit will produce a ΔA_{800} of 0.001 per min at pH 7.5 at 25 °C, using a suspension of yeast as substrate in a 3 mL reaction mixture.

References

1. Bir, N., et al., *Prep. Biochem.* **25**, 171–181 (1995)
2. Jazwinski, S.M., *Meth. Enzymol.* **182**, 154–174 (1990)
3. van Burik, J.A., et al., *Med. Mycol* **36**, 299–303 (1998)
4. Phalip, V., et al., *Biotechnol. Lett.* **26**, 409–413 (2004)

S: 22-24/25

lyophilized powder, activity: ≥2,000 units/mg protein, Protein: ~20%

Partially purified, lyophilized powder containing potassium phosphate buffer salts and stabilizers

-20°C

L2524-10KU	10,000 units
L2524-25KU	25,000 units
L2524-50KU	50,000 units
L2524-200KU	200,000 units

lyophilized powder, activity: ≥200 units/mg solid

-20°C

L4025-25KU	25,000 units
L4025-50KU	50,000 units
L4025-100KU	100,000 units
L4025-250KU	250,000 units
L4025-1MU	1,000,000 units

partially purified powder, activity: ≥2,000 units/mg protein

Partially purified powder containing ammonium sulfate and stabilizer

composition

Protein ~20% (biuret)

2-8°C

L5263-25KU	25,000 units
L5263-50KU	50,000 units
L5263-200KU	200,000 units

Lyticase from *Oerskovia xanthineolytica*

[37340-57-1]

Purified recombinant β-(1,3)-glucanase preparation that is protease free.

References

1. DeSampiao, G., *Mol. Microbiol.* **34**, 247–256 (1999)

recombinant, expressed in *Escherichia coli*, lyophilized powder

vial of ≥500 units

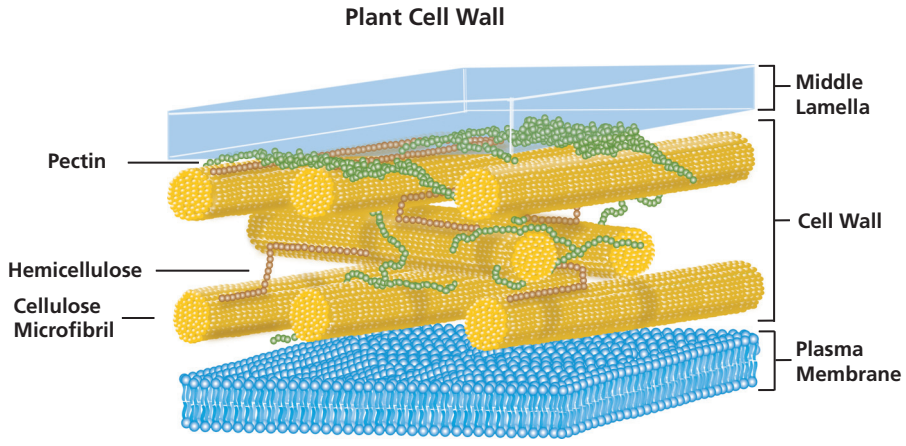
One unit will produce a ΔA_{800} of 0.001 per min at pH 7.5 at 25 °C, using a suspension of yeast as substrate in a 3 mL reaction mixture.

An exceptionally stable enzyme preparation with very low levels of nucleic acid and nuclease contamination.

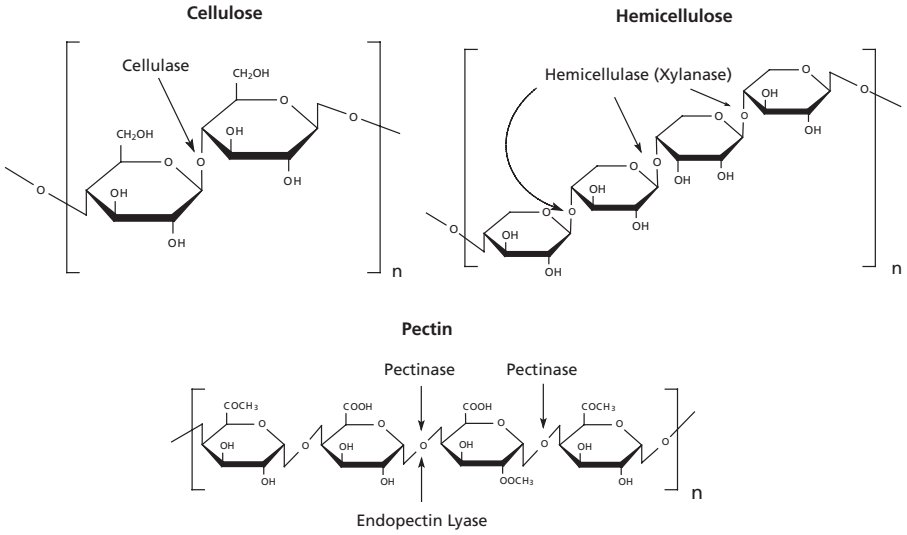
S: 22-24/25 -20°C

L4276-1VL	1 vial

Plant Cell Lysis and Protoplast Preparation



Plant cells are surrounded by a rigid, semi-permeable cell wall. The cell wall is comprised of mainly polysaccharides with some proteins and lipids. There are three main polysaccharide components of the cell wall. Cellulose is unbranched polymer of β-(1-4)-D-glycopyranosyl units associated in microfibril bundles. The microfibrils are cross-linked by hemicellulose (a branced polymer of β-(1-4)-D-xylopyranosyl units). This cross-linked structure is embedded in a matrix of pectin (primarily containing an β-(1-4)-polygalacturonic acid backbone, which can be randomly acetylated and methylated.



Reference

Carpita, N., and McCann, M., The cell wall. *In Biochemistry and Molecular Biology of Plants.* Buchanan, B., *et al.*, (Eds.) pp 52–108 (American Society of Plant Biologists, Rockville, MD, 2000).

Cellulase

Cellulase preparations are typically mixtures of enzymes containing high cellulase activity with some hemicellulase activity. These enzyme mixtures are capable of degrading cellulose, mannans, xylans, galactomannans, pectins, and other polysaccharides.

Cellulase from *Aspergillus* sp.

[9012-54-8] E.C. 3.2.1.4

activity: ≥1000 U/g

produced by submerged fermentation of a genetically modified *Aspergillus* microorganism

A product of Novozyme Corp.

R: 42 S: 23-24-36/37 EC No. 232-734-4 2-8°C

C2605-50ML	50 mL
C2605-250ML	250 mL

Cell Wall Degrading Enzyme Complex from *Aspergillus* sp.

Lysing Enzyme from *Aspergillus* sp.

Multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase

density..... ~1.2 g/mL, 25 °C

Viscozyme®

A product of Novozyme Corp.

2-8°C

V2010-50ML	50 mL
V2010-250ML	250 mL

Enzymes for Cell Lysis and Protoplast Preparation

Cellulase cont'd

Cellulase from *Aspergillus niger*

1,4-(1,3:1,4)-β-D-Glucan 4-glucano-hydrolase
[9012-54-8] E.C. 3.2.1.4; EC No. 2327344

powder, activity: ≥0.3 units/mg solid

One unit will liberate 1.0 µmole of glucose from cellulose in one hr at pH 5.0 at 37 °C (2 hr incubation time).

R: 42 S: 22-24-36/37 EC No. 232-734-4 2-8°C

C1184-5KU	5,000 units
C1184-25KU	25,000 units
C1184-100KU	100,000 units

Cellulase from *Trichoderma reesei* ATCC 26921

1,4-(1,3:1,4)-β-D-Glucan 4-glucano-hydrolase
[9012-54-8] E.C. 3.2.1.4

liquid, activity: ≥700 U/g

Produced by submerged fermentation of a selected strain of the fungus *Trichoderma reesei* and catalyzes the breakdown of cellulose into glucose, cellobiose, and higher glucose polymers.

density..... 1.2 g/mL, 25 °C

A product of Novozyme Corp.

R: 42 S: 23-24-36/37 EC No. 232-734-4 2-8°C

C2730-50ML	50 mL

lyophilized powder, activity: ≥1 unit/mg solid

One unit will liberate 1.0 µmole of glucose from cellulose in one hr at pH 5.0 at 37 °C (2 hr incubation time).

R: 42 S: 22-24-36/37 EC No. 232-734-4 2-8°C

C8546-2.5KU	2,500 units
C8546-5KU	5,000 units
C8546-10KU	10,000 units

Cellulase from *Trichoderma viride*

1,4-(1,3:1,4)-β-D-Glucan 4-glucano-hydrolase
[9012-54-8] E.C. 3.2.1.4; EC No. 2327344
Onozuka RS

powder, activity: ≥5,000 units/g solid

Manufactured by Yakult

One unit will liberate 1.0 µmole of glucose from cellulose in one hour at pH 5.0 at 37 °C (2 hr incubation time).

R: 42 S: 22-24-36/37 EC No. 232-734-4 2-8°C

C0615-1G	1 g

Cellulase from *Trichoderma viride*

1,4-(1,3:1,4)-β-D-Glucan 4-glucano-hydrolase
[9012-54-8] E.C. 3.2.1.4; EC No. 2327344

composition

Protein ~50% (biuret)

R: 42 S: 22-24-36/37 EC No. 232-734-4

plant cell culture tested, activity: 3–10 units/mg solid

contains lactose and glucose

One unit will liberate 1.0 µmole of glucose from cellulose in one hour at pH 5.0 at 37 °C (2 hr incubation time).

2-8°C

C1794-5KU	5,000 units
C1794-10KU	10,000 units

crude powder, activity: 3–10 units/mg solid

One unit will liberate 1.0 µmole of glucose from cellulose in one hour at pH 5.0 at 37 °C (2 hr incubation time).

2-8°C

C9422-5KU	5,000 units
C9422-10KU	10,000 units

Driselase from *Basidiomycetes* sp.

[85186-71-6]

Crude powder containing laminarinase, xylanase, and cellulase.

powder, Protein: ~15%

EC No. 286-055-3 –20°C

D9515-1G	1 g
D9515-5G	5 g
D9515-25G	25 g

Pectinase and Pectolyase

Pectinase catalyzes the random hydrolysis of α-(1-4)-D-galactosiduronic linkages in pectin and other galacturonans. Pectolyase catalyzes the eliminative cleavage of (1-4)-α-D-galacturonan methyl esters to give oligosaccharides with 4-deoxy-6-O-methyl-α-D-galact-4-enuronosyl groups at their non-reducing ends.

Pectinase from *Aspergillus aculeatus*

activity: ≥26,000 units/mL

Highly active pectolytic enzyme preparation produced by a selected strain of *Aspergillus aculeatus*

A product of Novozyme Corp.

2-8°C

P2611-50ML	50 mL
P2611-250ML	250 mL

Pectinase from *Aspergillus niger*

Pectolytic enzyme preparation produced from a selected strain of *Aspergillus niger*: contains mainly pectin transeliminase, polygalacturonase, and pectinesterase and small amounts of hemicellulases and cellulases.

A product of Novozyme Corp.

2-8°C

P2736-50ML	50 mL
P2736-250ML	250 mL

Pectinase and Pectolyase cont'd

Pectinase solution from *Aspergillus niger*

Polygalacturonase solution from *Aspergillus niger*; Poly-(1,4-α D-galacturonide) glycanohydrolase
[9032-75-1] E.C. 3.2.1.15

Used in plant protoplast preparation to digest cell wall prior to organelle isolation.

Solution in 40% glycerol

References

1. Nishimura, M., et al., Preparation of protoplasts from plant tissues *Meth. Enzymol.* **148**, 27–34 (1987)
2. Graham, J.M., and Rickwood, D., ed., Subcellular Fractionation, *A Practical Approach*, Oxford Univ. Press (New York: 1997), 256–258

EC No. 232-885-6

aqueous glycerol solution, activity: ≥5 units/mg protein (Lowry)

One unit will liberate 1.0 µmole of galacturonic acid from polygalacturonic acid per min at pH 4.0 at 25 °C.

2-8°C

P4716-5KU	5,000 units
P4716-10KU	10,000 units
P4716-25KU	25,000 units
P4716-100KU	100,000 units

plant cell culture tested, aqueous glycerol solution, activity: ≥5 units/mg protein (Lowry)

One unit will liberate 1.0 µmole of galacturonic acid from polygalacturonic acid per min at pH 4.0 at 25 °C.

2-8°C

P0690-10KU	10,000 units
P0690-25KU	25,000 units

Pectolyase from *Aspergillus japonicus*

E.C. 3.2.1.15

Reported to contain two types of pectinase, endopolygalacturonase (EC 3.2.1.15), endo-pectin lyase (EC 4.2.2.10) and a maceration stimulating factor.

Used in plant protoplast preparation to digest cell wall prior to organelle isolation.

Lyophilized powder containing lactose

References

1. Nishimura, M., et al., Preparation of protoplasts from plant tissues. *Meth. Enzymol.* **148**, 27–34 (1987)
2. Graham, J.M., and Rickwood, D., ed., *Subcellular Fractionation, A Practical Approach,* Oxford Univ. Press (New York: 1997), 256–258

lyophilized powder, activity: ≥0.3 units/mg solid

One unit will liberate 1.0 µmole of galacturonic acid from polygalacturonic acid per min at pH 5.5 at 25 °C.

S: 22-24/25 2-8°C

P3026-100MG	100 mg
P3026-250MG	250 mg
P3026-1G	1 g

plant cell culture tested, lyophilized powder
activity: ≥0.3 unit/mg solid
composition

Protein ~60% (Lowry)

One unit will liberate 1.0 µmole of galacturonic acid from polygalacturonic acid per min at pH 5.5 at 25 °C.

2-8°C

P5936-100MG	100 mg
P5936-250MG	250 mg
P5936-1G	1 g

aseptically filled, lyophilized powder, activity: ≥2 units/mg solid

Prepared from P3026

One unit will liberate 1.0 µmole of galacturonic acid from polygalacturonic acid per min at pH 5.5 at 25 °C.

2-8°C

P5431-25MG	25 mg

Pectinase from *Rhizopus* sp.

Macerozyme R-10; Polygalacturonase; Poly-(1,4-α-D-galacturonide) glycanohydrolase
[9032-75-1] E.C. 3.2.1.15; EC No. 2328856

Used in plant protoplast preparation to digest cell wall prior to organelle isolation.

One unit will liberate 1.0 µmole of galacturonic acid from polygalacturonic acid per min at pH 4.0 at 25 °C.

References

1. Graham, J.M., and Rickwood, D., ed., *Subcellular Fractionation, A Practical Approach*, Oxford Univ. Press (New York: 1997), 256–258
2. Nishimura, M., et al., Preparation of protoplasts from plant tissues. *Meth. Enzymol.* **148**, 27–34 (1987)

EC No. 232-885-6

crude powder, activity: 400–800 units/g solid

-20°C

P2401-500UN	500 units
P2401-1KU	1,000 units
P2401-5KU	5,000 units

plant cell culture tested, crude powder
activity: 400–800 units/g solid

-20°C

P4300-1KU	1,000 units
P4300-5KU	5,000 units

Enzymes for Cell Lysis and Protoplast Preparation

Mammalian Cell Permeabilization

Tetanolysin from *Clostridium tetani*

Cholesterol-binding toxin used to permeabilize cellular membranes to enhance the entry of macromolecules into the interior of the cell. Pores induced reported to be in the range of 20–50 nm.

mol wt 55 kDa

References

1. Haque, A., et al., *Infect. Immun.* **60**, 71 (1992)

Hemolytic activity of tetanolysin is determined using 2.5% rabbit red blood cells at 37 °C for 40 min.

Prepared by a modification of the method of Haque, *et al*.

When reconstituted with 100 µL of sterile water the concentration is 1 µg/µL in 40 mM sodium phosphate buffer, pH 7.2, containing 200 mM NaCl.

Single band by SDS-PAGE.

S: 22-28-36/37/39-45 2-8°C

T5319-100UG	100 µg

α-Hemolysin from *Staphylococcus aureus*

α-Toxin
[94716-94-6]

lyophilized powder, Protein: ~60%, activity: ≥10,000 units/mg protein

α-Hemolysin is a 33 kDa extracellular protein secreted by most strains of pathogenic *Staphylococcus aureus*. It is selectively hemolytic and has a marked preference for rabbit red blood cells. It induces dermonecrosis, spastic muscle paralysis, and it is lethal for laboratory animals. The toxin must be in the monomeric form to initially bind to a membrane and specific receptors are not required for binding. Upon binding to biological membranes and/or artificial membranes, self-oligomerization occurs, resulting in ring structures (hexameric aggregates) believed to represent transmembrane pores, which are permeable to ions and small metabolites.

It is thought that α-hemolysin stimulates cellular phospholipases and induces a Ca^{2+} influx that can result in membrane disruption, leakage of cytoplasmic components, impaired membrane permeability, and osmotic lysis of the cells.

contains sodium citrate buffer as balance

Package size based on protein content

One hemolytic unit will cause 50% lysis of a 1% suspension of rabbit red blood cells in phosphate buffered saline, pH 7.0, containing 1% bovine serum albumin after 30 min at 37 °C followed by refrigeration for 30 min at 4 °C.

References

1. Thelestam, M., and Blomqvist, L., Staphylococcal alpha toxin–recent advances. *Toxicon* **26**, 55–65 (1988)
2. Fink, D., et al., Staphylococcus aureus alpha-toxin activates phospholipases and induces a Ca^{2+} influx in PC12 cells. *Cell. Signal.* **1**, 387–393 (1989)

2-8°C

H9395-.5MG	0.5 mg
H9395-1MG	1 mg

Streptolysin O from *Streptococcus pyogenes*

[98072-47-0]

Thiol-activated toxin that permeabilizes animal cell membranes. The protein binds as a monomer to membrane cholesterol and subsequently polymerizes into large arc- and ring-shaped structures surrounding pores of >12 nm.

Permeabilizes membranes to permit cellular uptake of large or charged molecules.

References

1. Raufman, J.P., et al., *Biochim. Biophys. Acta* 1357, 73–80 (1997)
2. Alouf, J.E., *Pharmacol. Ther.* **11**, 661 (1980)
3. Bhakdi, S., et al., *Infect. Immun.* **46**, 394 (1984)
4. Palmer, M., et al., *Biochemistry* **37**, 2378–2383 (1998)
5. Wagner, A.C. and Williams, J.A., *Pancreas* **11**, 236–240 (1995)

EC No. 308-500-3

lyophilized powder, Protein: ~3%, activity: 25,000–50,000 U/vial

Lyophilized powder containing Tris buffer salts, sodium azide, PMSF, and EDTA.

native mol wt 69 kDa

One unit will cause 50% lysis of 2% red blood cell suspensions in phosphate buffered saline, pH 7.4, after incubation at 37 °C for 30 min.

2-8°C

S5265-25KU	25,000 units

γ-irradiated

Prepared from S5265

One unit will cause 50% lysis of 2% red blood cell suspensions in phosphate buffered saline, pH 7.4, after incubation at 37 °C for 30 min.

2-8°C

S0149-25KU	25,000 units

Proteases for Mitochondria Isolation

Mitochondria isolation is commonly utilized for apoptosis studies.[1] Such studies are of central importance for the investigation of a number of major debilitating diseases including Parkinson's disease and cancer.[2,3] In addition, mitochondrial protein isolation is of importance in proteome studies.[4,5]

Different procedures are required for mitochondria from "soft" tissues such as liver or brain, and from "hard" tissues such as skeletal muscle or heart muscle. The "soft" tissues are extracted in the presence of delipidated BSA that removes free fatty acids present in the tissue that cause uncoupling of respiration in the mitochondria.[6] EGTA is also present in the buffer to chelate Ca^{2+} ions that cause mitochondrial swelling.

"Hard" tissues cannot be homogenized easily without pretreatment with a protease to promote breakdown of the cellular structure. The myofibrils in skeletal muscle tend to give a gelatinous consistency to the homogenate in non-ionic media (isotonic sucrose) and thus must be isolated in an ionic medium such as 100 mM MOPS, pH 7.5, containing 550 mM KCl and 5 mM EGTA.[7]

Mitochondria can be prepared easily from animal tissues by a simple method of homogenization followed by low (600 × *g*) and high speed (11,000 × *g*) centrifugation.[8] The final pellet represents a crude mitochondrial fraction that may be used as the basis for further experiments. For a more purified "heavy" mitochondrial fraction that will be enriched in mitochondria as opposed to lysosomes and peroxisomes that normally contaminate this fraction, the low and high speed centrifugation steps can be changed to 1,000 × *g* and 3,500 × *g*, respectively.[6]

Assessment of the mitochondrial inner membrane integrity can be accomplished by testing of the electrochemical proton gradient ($\Delta\Psi$) of the inner mitochondrial membrane.[9] This may be achieved by measuring the uptake of the fluorescent carbocyanine dye JC-1 (Cat. No. T4069) into the mitochondria.[10,11]

The outer membrane integrity may be measured by observing cytochrome c oxidase activity (using the Cytochrome c Oxidase Assay Kit, Cat. No. CYTOCOX1). This kit measures the activity in the presence and absence of the detergent n-dodecyl β-D-maltoside, and the ratio of the two activities provides a measure of the integrity of the outer membrane.

References
1. Rampino, N., et al., *Science*, **275**, 9679 (1997).
2. Wallace, D.C., *Novartis Foundation Symposium*, **235**, 247 (2001).
3. Colin A. and Seamus M.J., *Trends in Biochem. Sci.*, **26**, 390 (2001).
4. Lopez M.F., et al, *Electrophoresis*, **21**, 3427 (2000).
5. Rabilloud, T., et al, *Electrophoresis*, **19**, 1006 (1998).
6. Graham, J.M., in Methods in Molecular Biology, *Biomembrane Protocols*, Graham, J.M. and Higgins, J.A. (Eds.), pp 29–57(Humana Press, 1993).
7. Lee, C.P., Biochem. *Biophys. Acta,* **1271**, 21 (1995).
8. Storrie, B. and Madden, E.A., *Methods Enzymol.*, **182**, 203 (1990).
9. Gross, A., et al., *J. Biol. Chem.*, **274**, 1156 (1999).
10. Reers, M., et al., *Biochem.*, **30**, 4480 (1991).
11. Salvioli, S., et al., *FEBS Letts.*, **411**, 77 (1997).

Papain from papaya latex

Papainase
[9001-73-4] E.C. 3.4.22.2

A cysteine protease that cleaves peptide bonds of basic amino acids, leucine, or glycine.

pH optimum 6.0–7.0

Also hydrolyzes esters and amides.

Used to produce Fab fragments of antibodies.[1] Also used for cell dissociation since it has been shown to be more effective and less damaging with certain tissues.[2,3]

mol wt 21 kDa

One unit will hydrolyze 1.0 µmole of BAEE per min at pH 6.2 at 25 °C.

Lit. cited:
1. Y. Ozari, J. Jagur-Grodzinski, *J. Chem. Soc. Chem. Commun*. 295 (1974)
2. M.A. Andrews, *Carbohydr. Res.* **194**, 1 (1989)
3. H. Cai et al., *Anal. Chem.* **70**, 580 (1998)

References
4. Dreyfus, Cheryl F., Black, Ira B., and, P. Michael Conn, ed., *Methods in Neuroscience,* Academic Press, Inc (San Diego: 1990), **2**, 10
5. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY: 1988), 626–628
6. Townes-Anderson, E., et al., Rod Cells Dissociated from Mature Salamander Retina: Ultrastructure and Uptake of Horseradish Peroxidase. *J. Cell Biol.* **100**, 175 (1985)

lyophilized powder, activity: ≥10 units/mg protein ($E^{1\%}_{280}$)

Lyophilized powder containing sodium chloride and sodium acetate

2× Crystallized

References
1. Fruton, J.S., *Adv. Enzymol.* **53**, 239 (1982)
2. Glazer, A.N., Smith, E.L., *The Enzymes* (1971), **3**, 501
3. Arnon, R., *Meth. Enzymol.* **19**, 226 (1970)

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-627-2 Light sensitive
RTECS # RU4950000 -20°C

P4762-25MG	25 mg
P4762-50MG	50 mg
P4762-100MG	100 mg
P4762-500MG	500 mg
P4762-1G	1 g
P4762-5G	5 g

Additional papain products, see *Page* 10 under Papain

Proteinase, bacterial

Nagarse; Subtilisin Carlsberg, bacterial
[9001-92-7] EC No. 2326424

E.C. 3.4.21.62

Type XXIV, activity: 7–14 units/mg solid, lyophilized powder

purified by crystallization

One unit will hydrolyze casein to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

Amino acid analysis and isoelectric focusing electrophoresis consistent with Subtilisin Carlsberg.

DNase, RNase ..... essentially free

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-642-4 RTECS # UK9540000 -20°C

P8038-50MG	50 mg
P8038-100MG	100 mg
P8038-250MG	250 mg
P8038-1G	1 g

Proteases for Mitochondria Isolation

Proteinase K from *Tritirachium album*

Endopeptidase K
[39450-01-6] E.C. 3.4.21.64; EC No. 2544578

Proteinase K is a stable and highly reactive serine protease.[1] Evidence from crystal and molecular structure studies indicates the enzyme belongs to the subtilisin family with an active-site catalytic triad (Asp^{39}-His^{69}-Ser^{224}). It is stable in a broad range of environments: pH, buffer salts, detergents (SDS), and temperature. In the presence of 0.1–0.5% SDS, proteinase K retains activity and will digest a variety of proteins and nucleases in DNA preparations without compromising the integrity of the isolated DNA.[2] Due to its broad specificity and activity in the presence of detergents, proteinase K has also been used to remove endotoxins bound to cationic proteins such as lysozyme and ribonuclease A.[3]

Useful for the proteolytic inactivation of nucleases during the isolation of DNA and RNA.

mol wt 28.93 kDa

References

1. Ebling, W., et al., *Eur. J. Biochem.* **47**, 91 (1974)
2. Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY: 1982)
3. Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.*, Humana Press Inc. (Towanam NJ: 1993), **16**, 306

lyophilized powder, activity: ≥30 units/mg protein

One unit will hydrolyze urea-denatured hemoglobin to produce color equivalent to 1.0 µmole of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

DNase.....<30 Kunitz units/mg solid
RNase.....<0.003 Kunitz units/mg solid

References

1. Buschmann, A., et al., *Biochem. Biophys. Res. Commun.* **253**, 693 (1998)
2. Kristjansson MM, et al., *Eur. J. Biochem.* **260**, 752 (1999)

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 254-457-8 -20°C

P6556-5MG	5 mg
P6556-10MG	10 mg
P6556-25MG	25 mg
P6556-100MG	100 mg
P6556-500MG	500 mg
P6556-1G	1 g

Subtilisin A

Protease from *Bacillus* sp.; Alkaline Protease; Proteinase from *Bacillus licheniformis*; Subtilisin Carlsberg; Subtilo peptidase A
[9014-01-1] E.C. 3.4.21.62; EC No. 2327522

Type VIII, lyophilized powder, activity: 7–15 units/mg solid

purified by crystallization

One unit will hydrolyze casein to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

DNase, RNase.....essentially free

References

1. Guntelberg, A.V. and Ottesen, M., *Comp. rend. Trav. Lab.* Carlsberg **29**, 36 (1954)

A product of Novozyme Corp.

R: 37/38-41-42 S: 22-24-26-36/37/39 EC No. 232-752-2 Hygroscopic
RTECS # CO9550000 -20°C

P5380-25MG	25 mg
P5380-100MG	100 mg
P5380-250MG	250 mg
P5380-1G	1 g

Trypsin from porcine pancreas

[9002-07-7] E.C. 3.4.21.4; EC No. 2326508

mol wt 23.8 kDa

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25° C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

One BTEE unit = 320 ATEE units

Type IX-S, lyophilized powder, activity: 13,000–20,000 BAEE units/mg protein

chymotrypsin.....≤1 units/mg protein

R: 36/37/38-42 S: 22-24-26-36/37 EC No. 232-650-8 Moisture sensitive
RTECS # YN5075000 -20°C

T0303-1G	1 g
T0303-10G	10 g

Additional trypsin products, see *Page* 11 under Trypsin

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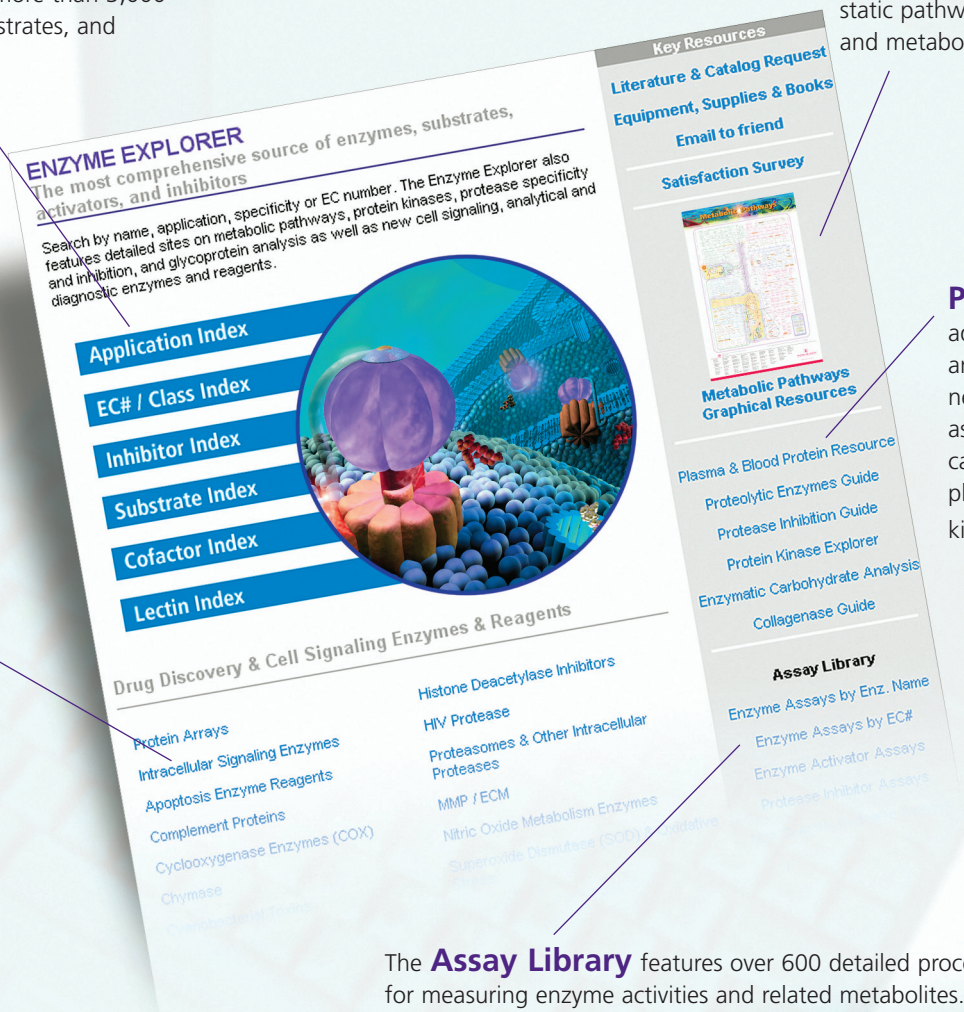

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