For life science research only.
Not for use in diagnostic procedures.

Collagenases
from *Clostridium histolyticum*

Version: 21
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Clostridiopeptidase A

Cat. No. 10 103 578 001	Collagenase A 100 mg
Cat. No. 11 088 807 001	Collagenase B 100 mg
Cat. No. 11 088 858 001	Collagenase D 100 mg
Cat. No. 10 103 586 001	Collagenase A 500 mg *Not available in US*
Cat. No. 11 088 815 001	Collagenase B 500 mg *Not available in US*
Cat. No. 11 088 866 001	Collagenase D 500 mg *Not available in US*
Cat. No. 11 088 793 001	Collagenase A 2.5 g
Cat. No. 11 088 831 001	Collagenase B 2.5 g
Cat. No. 11 088 882 001	Collagenase D 2.5 g

Store lyophilizates at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Collagenase A	Lyophilized, nonsterile	10 103 578 001	1 vial, 100 mg
			10 103 586 001	1 vial, 500 mg
			11 088 793 001	1 vial, 2.5 g
1	Collagenase B	Lyophilized, nonsterile	11 088 807 001	1 vial, 100 mg
			11 088 815 001	1 vial, 500 mg
			11 088 831 001	1 vial, 2.5 g
1	Collagenase D	Lyophilized, nonsterile	11 088 858 001	1 vial, 100 mg
			11 088 866 001	1 vial, 500 mg
			11 088 882 001	1 vial, 2.5 g

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizates are stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	Collagenase A	Store dry at +2 to +8°C. ⚠ ***Keep protected from light.***
1	Collagenase B	
1	Collagenase D	

Storage Conditions (Working Solution)

Store reconstituted solution at −15 to −25°C for up to one week.
⚠ ***Avoid repeated freezing and thawing since activity decreases after reconstitution.***

Reconstitution

Reconstitute Collagenase A, B, or D in any balanced salt solution, such as HBSS (Hank's Balanced Salt Solution).
⚠ ***Reconstitute only the amount of lyophilizate needed for immediate use.***

Composition of selected balanced salt solutions[1]

Reagent	Ringer	Tyrode	Gey	Earle	Puck	Hanks	Dulbecco (PBS)
NaCl	9.00	8.00	7.00	6.80	8.00	8.00	8.00
KCl	0.42	0.20	0.37	0.40	0.40	0.40	0.20
$CaCl_2$	0.25	0.20	0.17	0.20	0.012	0.14	0.10
$MgCl_2 \times 6 H_2O$	–	0.10	0.21	–	–	0.10	0.10
$MgSO_4 \times 7 H_2O$	–	–	0.07	0.10	0.154	0.10	–
$Na_2HPO_4 \times 12 H_2O$	–	–	0.30	–	0.39	0.12	2.31
$NaH_2PO_4 \times H_2O$	–	0.05	–	0.125	–	–	–
KH_2PO_4	–	–	0.03	–	0.15	0.06	0.20
$NaHCO_3$	–	1.00	2.27	2.20	–	0.35	–
Glucose	–	1.00	1.00	1.00	1.10	1.00	–
Phenol red	–	–	–	0.05	0.005	0.02	–
Atmosphere	air	air	95% air/ 5% CO_2	95% air/ 5% CO_2	air	air	air

[1] Amounts are given as grams per liter of solution.

1.3. Additional Equipment and Reagent required

For tissue dissociation of minced tissue

- PBS, sterile or another balanced salt solution
- Filter membrane, 0.22 µm
- Nylon mesh or gauze
- Scalpel

1.4. Application

Collagenase from *C. histolyticum* is widely used for the:
- Disaggregation of many types of tissues, such as lung, heart, muscle, bone, adipose, liver, kidney, cartilage, mammary gland, placenta, blood vessels, brain, and all types of tumors.
- Preparation of single cell suspensions for the establishment of primary cell culture systems.
- Preparation of cells from many types of tissue, such as hepatocytes, adipocytes, pancreatic islets, epithelial cells, muscle cells, endothelial cells, etc.

ⓘ *Suitability of each lot of the enzyme for disruption of a particular tissue must be determined empirically.*

2. How to Use this Product

2.1. Before you Begin

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Two types of procedures are commonly used:

- Mincing tissue and incubating the pieces in a collagenase solution with mild agitation. Cells are gradually released from the tissue during the collagenase treatment.
- Perfusing an organ with the collagenase solution. Cells are gradually released into the perfusate or the tissue is then dissociated by mild mechanical treatment.

  For organ perfusion procedures, special products are available, such as Collagenase H for hepatocyte isolation and Collagenase P* for pancreatic islet isolation.*

Tissue dissociation of minced tissue

1. Dissolve the non-sterile, lyophilized enzyme in a balanced salt solution, and filter using a 0.22 µm filter membrane.

2. Wash the tissue in sterile PBS or another balanced salt solution.

3. Remove undesirable tissue, such as fat or necrotic material, and cut the remaining tissue with a scalpel into 1 to 3 mm cubes.

4. Add approximately 0.1% to 0.25% (w/v) collagenase solution.

   It is possible, but in most cases not necessary to add other enzymes, such as Pronase, hyaluronidase, elastase, or trypsin to the collagenase solution.*

5. Incubate at +37°C until disaggregation is completed.

6. Check for effective disaggregation.
   – If the cell suspension becomes viscous due to DNA release from digested cells, add DNase I* to alleviate this problem.
   – If necessary, separate undissociated fragments from single cells by collecting the supernatant after allowing the fragments to settle and add fresh enzyme solution to the tissue fragments.
   – The cell suspension can be passed through a nylon mesh or gauze to remove undigested fragments.

7. Centrifuge the supernatant(s) at 50 to 100 × *g* for approximately 3 minutes.
   – Resuspend the pellet in medium and seed as usual.

2.3. Parameters

Activator

Ca^{2+}

EC-Number

EC 3.4.24.3

Inhibition

EDTA, EGTA, Cys, His, DTT, 2-mercaptoethanol.
Collagenase is not inhibited by serum.

pH Optimum

pH 6.0 to 8.0

Specific Activity

>0.15 U/mg lyophilizate (collagenase activity)
The preparations contain other enzyme activities from which the following are routinely measured for each lot:

Collagenase	Additional enzyme activities
A	Normal balanced ratio of enzyme activities.
B	Normal to high collagenase activity and higher than normal clostripain activity (usually >10 U/mg).
D	Normal to high collagenase activity and very low tryptic activity (usually <0.2 U/mg).

Collagenase A, B, and D contain different ratios of the various proteolytic activities:

Enzyme	Proteolytic activity
Clostripain activity	1 U catalyzes the hydrolysis of 1 µmol N-α-benzoyl-L-arginine ethyl ester (BAEE) per minute at +25°C and pH 7.6 after activation with 1 mM calcium acetate and 2.5 mM dithiothreitol.
Tryptic activity	With BAEE as substrate: 1 U is that enzyme activity which hydrolyzes 1 µmol BAEE in 1 minute at +25°C and pH 7.6.
Protease activity	1 U is that protease activity which is causing an absorption increase of 0.001 in 1 minute at +25°C in the standard azocoll test.

Unit Definition

1 U is the activity which liberates in 1 minute at +25°C 1 µmol 4-phenyl-azobenzyl-oxycarbonyl-L-prolyl-L-leucine from 4-phenyl-azobenzyl-oxycarbonyl-L-prolyl-L-leucyl-glycyl-L-prolyl-D-arginine (substrate according to Wünsch) under assay conditions (Wünsch E, Heidrich HG, 1963).
Collagenase is assayed in Wünsch units (1 µmol of product formed per minute at +25°C with Wünsch substrate). Frequently, collagenase activities are given in Mandl units (1 µmol leucine liberated from collagen in 5 hours at +37°C).
Unfortunately, there is no consistent conversion factor between the two units of activity, since the Mandl unit depends, in part, on the concentration of contaminating proteases in the collagenase preparation, an indefinable variable. A purer collagenase preparation would result in a lower specific activity in Mandl units than a crude preparation. *Clostridium* preparations typically provide conversion factors of approximately 1:1,800, for example, a particular lot of *Clostridium* collagenase has approximately 0.15 Wünsch U/mg and 250 Mandl U/mg.

Working Concentration

Approximately 1 mg/ml (0.1%, w/v).

3. Additional Information on this Product

3.1. Test Principle

Bacterial collagenase, or more precisely clostridiopeptidase A, is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most frequently a neutral amino acid. Such sequences are found in high frequency in collagen, but only rarely in other proteins. While many proteases can hydrolyze single-stranded, denatured collagen polypeptides, clostridiopeptidase A is unique among proteases in its ability to attack and degrade the triple-helical native collagen fibrils commonly found in connective tissue.
Purified clostridiopeptidase A alone is usually inefficient in dissociating tissues due to incomplete hydrolysis of all collagenous polypeptides. Its activity is limited against high concentrations of non-collagen proteins and other macromolecules found in the extracellular matrix.
The collagenase most commonly used for tissue dissociation is a crude preparation from *Clostridium histolyticum* containing clostridiopeptidase A, in addition to a number of other proteases, polysaccharidases, and lipases. Crude collagenase is apparently ideally suited for tissue dissociation, because it contains the enzyme required to attack native collagen, in addition to the enzymes which hydrolyze the other proteins, polysaccharides and lipids in the extracellular matrix of tissues.
Collagenase A, B, and D are prepared from the extracellular culture filtrate of *Clostridium histolyticum*. These crude preparations contain collagenase and other proteases, including clostripain, a trypsin-like activity and a neutral protease. This mixture of enzyme activities makes crude collagenases ideally suited for gentle dissociation of tissue to generate single cells. Collagenase A, B, and D contain different ratios of the various proteolytic activities. This allows for selection of the preparation best suited for disaggregation of a particular tissue.

Preparation

Collagenase A, B, and D are prepared from *C. histolyticum* cultures by filtration, ammonium sulfate precipitation, dialysis, and lyophilization.

3.2. References

- Wünsch E, Heidrich HG. On the quantitative determination of collagenase. Hoppe Seylers Z Physiol Chem. 1963;333:149-151.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DNase I	100 mg, *Not available in US*	11 284 932 001
Collagenase H	100 mg	11 074 032 001
	500 mg	11 074 059 001
	2.5 g	11 087 789 001
Collagenase P	100 mg	11 213 857 001
	500 mg, *Not available in US*	11 213 865 001
	1 g	11 249 002 001
	2.5 g	11 213 873 001
Pronase	1 g	10 165 921 001
	5 g	11 459 643 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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