Print

Roche

Collagenase A

From *Clostridium histolyticum*
Clostridiopeptidase A, EC 3.4.24.3 Lyophilizate

Cat. No. 10 103 578 001 100 mg
Cat. No. 10 103 586 001 500 mg
Cat. No. 11 088 793 001 2.5 g

Collagenase B

From *Clostridium histolyticum*
Clostridiopeptidase A, EC 3.4.24.3 Lyophilizate

Cat. No. 11 088 807 001 100 mg
Cat. No. 11 088 815 001 500 mg
Cat. No. 11 088 831 001 2.5 g

Collagenase D

From *Clostridium histolyticum*
Clostridiopeptidase A, EC 3.4.24.3 Lyophilizate

Cat. No. 11 088 858 001 100 mg
Cat. No. 11 088 866 001 500 mg
Cat. No. 11 088 882 001 2.5 g

Version 18.0
Content version: July 2010

Store at +2 to +8°C
Store dry and protected from light!

1. What this Product Does

Contents

Lyophilizate, non-sterile

Preparation

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

Product Characteristics

Specific Activity	>0.15 U/mg lyophilizate (collagenase activity): 1 U is the activity which liberates in 1 min 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 (1).
Inhibitors	EDTA, EGTA, Cys, His, DTT, 2-mercaptoethanol. Collagenase is not inhibited by serum
Activators	Ca^{2+}
pH Optimum	6.0 – 8.0.

Additional Enzyme Activities

The preparations contain other enzyme activities, from which the following are routinely measured for each lot:

Collagenase A	Normal balanced ratio of enzyme activities.
Collagenase B	Normal to high collagenase activity and higher than normal clostripain activity (usually >10 U/mg).
Collagenase 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	Activity
Clostripain	1 U catalyzes the hydrolysis of 1 μmol N-α-benzoyl-L-arginine ethylester (BAEE) per min 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 min 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 min at 25°C in the standard azocoll test.

Application

Collagenase from *C. histolyticum* is now widely used for the disaggregation of all kind of tissues (*e.g.,* lung, heart, muscle, bone, adipose tissue, liver, kidney, cartilage, mammary gland, placentae, blood vessels, brain, all kind of tumors) and for the preparation of single cell suspensions for the establishment of primary cell culture systems.

Clostridium collagenase from Roche has been used to prepare cells from many types of tissue, *e.g.,* hepatocytes, adipocytes, pancreatic islets, epithelial cells, muscle cells, endothelial cells etc. (5-19). However, suitability of each lot of the enzyme for disruption of a particular tissue should be determined empirically.

Storage and Stability

The lyophilizate is stable at +2 to +8°C, when stored dry and protected from light, until the expiration date printed on the label.

The reconstituted solution is stable at −15 to −25°C.

2. How to Use this Product

2.1 Before You Begin

Reconstitution

In any balanced salt solution (*e.g.,* HBSS) (see table 1).

Tab. 1: Composition of selected balanced salt solutions [1].

	Ringer [2]	Tyrode [3, 4]	Gey [5]	Earle [6]	Puck [7]	Hanks [8]	Dulbecco (PBS) [9, 10]
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
$MgC_2 \times 6\ H_2O$		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 ret				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.

2) Ringer, S. (1985) *J. Physiol.* (London) **18**, 425.

3) Tyrode, M. V. (1910) *Arch. Int. Pharmacodyn. Ther.* **20**, 205.

4) Parker, R. C. (1961) "*Methods of Tissue Culture*", 3 rd ed., p. 57, Harper (Hoeber), New York.

5) Gey, G. O. & Gey, M. K. (1936) *Am. J. Cancer* **27**, 55.

6) Earle, W. R. (1943) *J. Natl. Cancer Inst.* **4**, 165.

7) Puck, T. T., Cieciura, S. J. & Robinson, A. (1958) *J. Exp. Med.* 108, 945.

8) Hanks, J. H. & Wallace, R. E. (1949) *Proc. Soc. Exp. Biol. Med.* **71**, 196.

9) PBS, phosphate-buffered saline

10) Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167.

Working Concentration

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

Additional Reagents and Material Required

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

2.2 Procedure

Two types of procedures are commonly used. The first involves mincing tissue and incubating the pieces in a collagenase solution with mild agitation.

Cells are gradually released from the tissue during the collagenase treatment. The second involves perfusing an organ with the collagenase solution. Cells are gradually released into the perfusate or the tissue is then dissociated by mild mechanical treatment.

The following working instruction describes, as an example, a procedure for minced tissue:

❶	Dissolve the non-sterile, lyophilized enzyme in a balanced salt solution and filter sterilize through a 0.22 µm filter membrane.
❷	Wash the tissue in sterile PBS or another balanced salt solution.
❸	Remove undesirable tissue like fat or necrotic material and cut the remaining tissue with a scalpel into 1 – 3 mm cubes.
❹	Add collagenase solution [usually 0.1% to 0.25% (w/v)]. It is possible, but in most cases not necessary, to add other enzymes such as pronase*, hyaluronidase*, elastase* or trypsin* to the collagenase solution.
❺	Incubate at 37°C until disaggregation is complete.
❻	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 gaze to remove any undigested fragments.
❼	Centrifuge the supernatant(s) at 50 – 100 × *g* for about 3 min.
❽	Resuspend the pellet in medium and seed as usual. For organ perfusion procedures special products (collagenase H* for hepatocyte isolation, collagenase P* for pancreatic islet isolation) are available.

3. Additional Information on this Product

Background Information

Bacterial collagenase, or more accurately 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 (2–4).

Purified clostridiopeptidase A alone is usually inefficient in dissociating tissues due to incomplete hydrolysis of all collagenous polypeptides and its limited activity against the high concentrations of non-collagen proteins and other macromolecules found in the extracellular matrix.

The collagenase most commonly used for tissue dissociation is a crude preparation from C. histolyticum containing clostridiopeptidase A in addition to a number of other proteases, polysaccharidases and lipases.

Crude collagenase is apparently ideally suited for tissue dissociation since 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.

Unit Definition

Collagenase from Roche is assayed in Wünsch units (1µmol of product formed per minute at 25°C with Wünsch substrate [1]).

Frequently, collagenase activities are given in Mandl units (1 µmol leucine liberated from collagen in 5 h 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 actually give a lower specific activity in Mandl units than a crude preparation. Clostridium preparations typically give conversion factors of approx. 1:1800 (*e.g.,* a particular lot of Clostridium collagenase contained approx. 0.15 Wünsch U/mg and 250 Mandl U/mg).

References

1 Wünsch, E. & Heidrich, H. (1963) *Z. Physiol. Chem.* **333**, 149-159.
2 Seifter, S. & Harper, E. (1970) *Methods Enzymol.* **19**, 613-635.
3 Harper, E. (1980) *Ann. Rev. Biochem.* **49**, 1063-1078.
4 Peterkofsky, B. (1982) *Methods Enzymol.* **82**, 453-471.
5 Moldeús, P., Högberg, J. & Orrenius, S. (1978) *Methods Enzymol.* **52**, 60-71.
6 Hellerström, C. *et al.,* (1979) *Diabetes* **28**, 769-776.
7 Schlepper-Schäfer, J., Kolb-Bachofen, V. & Kolb, H. (1980) *Biochem. J.,* **186**, 827-831.
8 Eckel, J. & Reinauer, H. (1982) *Biochem. J.* **206**, 655-662.
9 Jones, B. E. & Yong, L. C. J. (1987) *In Vitro* **23**, 698-706.
10 Cardelli-Cangiano, P. *et al.,.* (1987) *J. Neurochem.* **49**, 1667-1675.
11 Innes, G. K., Fuller, B. J. & Hobbs, K. E. F. (1988) *In Vitro* **24**, 126-132.
12 Amory, B., Mourmeaux, J.-L. & Remacie, C. (1988) *In Vitro* **24**, 91-98.
13 Vandenberghe, Y. (1988) *In Vitro* **24**, 281-288.
14 Petzinger, E. *et al.,* (1988) *In Vitro* **24**, 491-499.
15 Lopez, M. P., Gomez-Lechon, M. J. & Castell, J. V. (1988) *In Vitro* **24**, 511-517.
16 Andersson, A., Korsgren, O. & Jansson, L. (1989) *Diabetes* **38**, 192-195.
17 Korsgren, O. *et al.,* (1989) *Diabetes* **38**, 209-212.
18 Martin, M., Remy, J. & Daburon, F. (1989) *J. Invest. Dermatol.* **93**, 497-500.
19 Lindsay, R. M. (1988) *J. Neurosci.* **8**, 2394-2405.

4. Supplementary Information

4.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered instructions labeled 1, 2, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Changes to the Previous Version

- Lot specific information is no longer shown in the label on the upper left-hand side of the Instruction for Use.
- Please refer to the Certificate of Analysis for more lot specific information.
- Revised regulatory disclaimer

4.3 Trademarks

All brands or product names are trademarks of their respective holders.

Regulatory Disclaimer

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

Contact and Support

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

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany