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Collagenase I, II, III, IV

Clostridiopeptidase A from *Clostridium histolyticum*

Product	Cat#	Package size	Enzyme Activities
Collagenase I	**C4255**	**100mg, 1g**	Normal balanced ratio of enzyme activities. Mixture of Collagenase, Clostripain as well as tryptic and proteolytic activities. Recommended for liver, lung, fat and adrenal cortex tissue. Its specific activity is 125 to 250 Mandl units per mg dry substance.
Collagenase II	**C4341**	**100mg, 1g**	Mixture of Collagenase, Clostripain as well as tryptic and proteolytic activities. Normal to high collagenase activity and higher than normal clostripain and trypsin activity. Recommended for the isolation of liver, bone, thyroid gland, and heart cells. Its specific activity is 125 to 250 Mandl units per mg dry substance.
Collagenase III - Premium grade with high specific activity	**C4346**	**>2000 PZ units**	High level Collagenase with normal collagenase activity but much lower proteolytic activity from Clostripain, Trypsin-like or Neutral protease. Recommended for mammary gland tissue. Its specific activity is >3000 Mandl units per mg dry substance.
Collagenase IV	**C4310**	**100mg, 1g**	Mixture of Collagenase, Clostripain as well as tryptic and proteolytic activities. With low tryptic, high collagenase and normal Clostripain activity. Recommended for Langerhans islet cells. Its specific activity is >900 Mandl units per mg dry substance.

Description

The cell structure of animal tissue is built up by a complex matrix of proteins (e.g., Collagen), glycoproteins, lipids, glycolipids and mucopolysaccharides. For the isolation of single cells or for the establishment of primary cell culture systems this complex matrix must be digested very carefully without irreversible damage of the cell surface or intra cellular structures. The treatment of tissue with Collagenase effectuates a careful and selective degradation of the inter cellular matrix without harming the ability of cells to grow.

Genaxxon bioscience Collagenase is a mixture of different proteolytic enzymes. For optimal results, a fine tuning of enzyme activities is necessary. It is for that reason four different Collagenase fractions are available with different ratios of enzyme activities (see list above).

Most often Collagenase IV is used together with other enzymes like Trypsin, Elastase or Hyaluronidase.

The normally in cell culture used Trypsin, respective Trypsin/EDTA does only affect the matrix very slowly and does irreversible impair cells.

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Background:

Bacterial collagenase (clostridiopeptidase A) is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most often a neutral amino acid. These sequences are found in high frequency in collagen, but only rarely in other proteins.

Collagenase I, II, III, IV 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 I, II, III, IV contain different ratios of the various proteolytic activities. This allows for selection of the preparation best suited for disaggregation of a particular tissue.

Activity:	>100 U/mg lyophilized Collagenase (µmoles L-leucine (equivalent liberated) / mg dissolved lyophilized enzyme.
Unit Definition:	One unit equals one micromole of L-leucine equivalents from collagen in 5 hours at 37°C and pH7.5 under the specified conditions.
Method:	A modification of the procedure of Mandl et al. (1953). Collagenase is incubated for 5 hours with native collagen. The extent of collagen breakdown is determined using the Moore and Stein (1948) colorimetric ninhydrin method.
Formulation:	lyophilized, non-sterile.
Preparation:	Collagenase I, II, III and IV are prepared from C. histolyticum cultures by filtration, ammonium sulphate precipitation, dialysis and lyophilisation.
Inhibitors:	EDTA, EGTA, Cys, His, DTT, 2-mercaptoethanol. Collagenase is not inhibited by serum.
Activators:	Ca2+
pH-Optimum:	6.0 – 8.0.
Source:	*Clostridium histolyticum*
Application:	Collagenase from *C. histolyticum* is widely used for the disaggregation of all kinds of tissues (e.g., lung, heart, muscle, bone, adipose tissue, liver, kidney, cartilage, mammary gland, placenta, 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 has been used to prepare cells from many types of tissue. However, suitability of each lot of the enzyme for disruption of a particular tissue should be determined empirically.
Reconstitution:	Reconstitute in water (100mg/mL) and dilute further in any balanced salt solution (e.g., HBSS or PBS).
Working concentration:	approximately 1mg/mL to 2mg/mL (0.1% to 0.2% (w/v))
Storage /	Lyophilisate is stable at +2°C to +8°C if stored at a dry and dark place (protect from light) for at least 2 years.
Stability:	The reconstituted solution should be stored at -20°C for 1 year. It is recommended to prepare aliquots from the reconstituted solution.

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PROCEDURES AND REQUIRED MATERIAL

General:

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.

Additional material: sterile PBS, or another balanced salt solution, filter membrane (0.22µm), nylon mesh or gaze.

PROTOCOL

Step	
Preparation of a working solution	
1	Dissolve the non-sterile lyophilised enzyme in water (appr. 100mg/mL). Dilute with a balanced salt solution (PBS or HBSS) and filter sterilise through a 0.22µm filter membrane. The working concentration should be approximately: 100 - 200 U/mL.
Tissue disaggregation	
1	Wash the tissue in sterile PBS or another balanced salt solution.
2	Remove undesirable tissue like fat or necrotic material and cut the remaining tissue with a scalpel into 1-3 mm cubes.
3	Add collagenase solution (appr. 0.1% to 0.25% (w/v) and medium (with or without serum). Sometimes it will be necessary to add other enzymes such as pronase, elastase, or additional trypsin.
4	Incubate at 37°C until disaggregation is complete (1-48 hours depending on tissue). Control of pH!
5	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.
Subcultivation of cells	
1	Centrifuge the tissue free medium at 50 – 100 x g for about 3 minutes. Discard supernatant(s).
2	Resuspend the pellet and wash cells in sterile PBS and medium. Control quality of cells, e.g., with a microscope.
3	Seed and cultivate as usual.

Application Notes

The working concentration of Collagenase should be (depending on the tissue) between 0.1% and 0.2% (w/v). This value is valid for an activity of about 160 Mandl units per mg dry substance. If the Collagenase batch does show another specific activity, the concentration must be adopted accordingly.

Collagenase is a natural, biological product and shows batch to batch fluctuations in the specific activity. For those reason adjustments in concentration, temperature and residence time might be necessary from batch to batch to optimize results.

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