

# Genaxxon BioScience

## Collagenase

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Clostridiopeptidase A from *Clostridium histolyticum*

Product	Cat#	Package size	Enzyme Activities
Collagenase I	C4255	100mg, 1g, 5g	Normal balanced ratio of enzyme activities.
Collagenase II	C4256	100mg, 1g, 5g	Normal to high collagenase activity and higher than normal clostripain <u>and</u> trypsin activity.
Collagenase III	C4257	100mg, 1g, 5g	Normal to higher collagenase activity and very low tryptic activity.
Collagenase IV	C4258	100mg, 1g, 5g	Selected low tryptic activity at high collagenase and normal clostripain activity.

<b>Activity:</b>	> 650 CDU/mg (CDU = Collagen Digesting Units).
<b>Unit Definition:</b>	One unit is defined as the amount of enzyme that causes the hydrolysis of bovine tendon collagen (Worthington CL) yielding a colour corresponding to 1 µmol L-leucine/mg collagenase (these units are sometimes termed "Mandl Units").
<b>Method:</b>	A modification of the procedure of Mandl et al. (1953). Collagenase and bovine tendon collagen are incubated together. The cleavage of the tendon collagen is measured by appearance of ninhydrin positive products. The exact procedure is published in Worthington Enzyme Manual.
<b>Formulation:</b>	lyophilized, non-sterile.
<b>Preparation:</b>	Collagenase I, II, III and IV are prepared from <i>C. histolyticum</i> cultures by filtration, ammonium sulphate precipitation, dialysis and lyophilisation.
<b>Additional enzyme activity:</b>	The preparations contain other enzyme activities of different amount. Collagenase I, II, III and IV contain different ratios of the various proteolytic activities (from clostripain, trypsin and collagenase).
<b>Inhibitors:</b>	EDTA, EGTA, Cys, His, DTT, 2-mercaptoethanol. Collagenase is not inhibited by serum.
<b>Activators:</b>	Ca <sup>2+</sup>
<b>pH-Optimum:</b>	6.0 – 8.0.
<b>Isoelectric point:</b>	5.4 – 6.2
<b>Optimal temp.</b>	37°C
<b>Source:</b>	<i>Clostridium histolyticum</i>
<b>Application:</b>	Collagenase from <i>C. histolyticum</i> is widely used for the disaggregation of all kind 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.
<b>Reconstitution:</b>	Reconstitute in water (100 mg/ml) and dilute further in any balanced salt solution (e.g. HBSS or PBS).
<b>Working concentration:</b>	approximately 1 mg/ml (0.1% w/v)
<b>Storage / Stability:</b>	Lyophilisate is stable at +4°C if stored at a dry and dark place (protect from light). The reconstituted solution should be stored at -20°C. It is recommended to prepare aliquots from the reconstituted solution.

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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.

## 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 (0.22 µm), nylon mesh or gaze.

## PROTOCOL

Step	
<b>Preparation of a working solution</b>	
1	Dissolve the non-sterile lyophilised enzyme in water (appr. 100 mg/ml). Dilute with a balanced salt solution (PBS or HBSS) and filter sterilise through a 0.22 µm filter membrane. The working concentration should be approximately 1 mg/ml (0.1% w/v).
<b>Tissue disaggregation</b>	
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.
<b>Subcultivation of cells</b>	
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.