

Technical Data Sheet

Collagenase

for cell biology

Order number: 1904 (Type I)

2275 (Type II)

2091 (Type IV)

Collagenase (also called Clostridiopeptidase A) is an enzyme derived from *Clostridium histolyticum*. Collagenases are endopeptidases that digest native collagen in triple helix region. They show specificity for the sequence R-Pro-(-X-Gly-Pro), where X is most often a neutral amino acid. Bacterial collagenases have a broader specificity than vertebrate collagenases. Bacterial collagenases can degrade both, water-insoluble native collagens and water-soluble denatured collagens. The extracellular matrix in animal tissue is a complex mixture of collagens and other extracellular matrix proteins like glycoproteins and proteoglycans. This matrix must be effectively broken down to isolate single cells, without alteration of cellular structures. Crude collagenase is a mixture of collagenases and other proteolytic enzymes. It contains a sulphhydryl protease, clostripain, a trypsin-like enzyme and an aminopeptidase. This combination of collagenolytic and proteolytic activities is effective at breaking down intercellular matrices, the essential part of tissue dissociation.

The most commonly used collagenase is a crude preparation from the extracellular culture filtrate of *Clostridium histolyticum*. Crude collagenase (ranging in molecular weight from 68-125 kDa). is classified into different types based on different ratios of various proteolytic activities. The difference in enzymatic ratios allow for selection of the collagenase type best suited for dissociation of a particular tissue. However, crude collagenases do exhibit lot to lot variability and the suitability for disruption of a particular tissue should be determined empirically.

- Type I:** Original balance of collagenase, caseinase, clostripain and tryptic activities. Suggested for epithelial, liver, lung and adrenal primary isolations.
- Type II:** Prepared to contain higher clostripain activity. Suggested for bone, heart, liver, thyroid and salivary primary cell isolation.
- Type IV:** Prepared to contain lower tryptic activity levels to limit damage to membrane proteins and receptors with normal to high collagenase activity. Suggested for pancreatic islet primary isolation.



Application

1. Reconstitution

Dissolve lyophilized powder in a balanced salt solution and sterilize by filtration through a 0.22 micron filter membrane. Filter with low protein binding properties like PES or PVDF should be used.

2. Dissociation

Wash the tissue in sterile PBS or other balanced salt solution. Remove undesirable tissues like fat or necrotic material and cut the tissue with a scalpel or scissors. Add collagenase solution. Crude collagenase is most often used at 0.1% - 0.5% (w/v) concentration or 50 – 100 U/ml. Dissociation efficiency is increased if the digest is supplemented with 3 - 5mM calcium chloride. Incubate at 37°C until disaggregation is complete.

Enzyme activity; unit definition

One **Collagen unit** liberates peptides from collagen equivalent in ninhydrin color to 1.0 µmol of leucine in 5 hours at pH 7.4 and 37°C in the presence of calcium ions.

One **Clostripain unit** hydrolyzes 1.0 µmol of N-Q-benzoyl-L-arginine ethyl ester (BAEE) per min at pH 7.6 and 25°C in the presence of DTT.

One **Tryptic unit** hydrolyzes 1.0 µmol of N-Q-benzoyl-L-arginine ethylester (BAEE) in one min at pH 7.6 and 25°C.

One **Protease unit** hydrolyzes casein to produce colore equivalent to 1.0 µmol tyrosine per 5 hours at pH 7.5 and 37°C.

Note: Ca²⁺ ions are required for enzyme activity. The activity is inhibited by metal chelating agents such as EDTA, cysteine, o-phenanthroline and α2-macroglobulin.

Storage

Store Collagenase powder at 2-8°C. Reconstituted solution should be stored at -20°C. Avoid repeated freezing and thawing.

Related products

1429	D-PBS (1X) w/o Calcium and Magnesium, pH 7.4 for cell biology
1264	Dimethyl sulfoxide for cell biology
1194	HEPES for cell biology
1210	L-Glutamine for cell biology
1229	Sodium hydrogen carbonate for cell biology
1243	Vitamin B12 for cell biology
1510	Penicillin/Streptomycin solution (in 0.85 % NaCl) for cell biology

JB16052019

