

Collagenase Type I

Cell Culture Tested

Product Code: TC211

Product Description :

Molecular Weight: 68,000 to 125,000

CAS No.: 9001-12-1

Synonym: Clostridiopeptidase A

E. C. No.: 3.4.24.3

Collagenase is an enzyme derived from the fermentation of *Clostridium histolyticum*. Collagenases are endopeptidases that digest native collagen in triple helix region. Collagenases have 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 in breaking down intercellular matrices, the essential part of tissue dissociation.

Activity:

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

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

One Tryptic unit is that enzyme activity which hydrolyzes 1.0μmole of (N-Q-benzoyl-L-arginine ethyl ester (BAEE) in one min at 25°C and pH 7.6.

One protease unit hydrolyzes casein to produce color equivalent to 1.0μmole tyrosine per 5 hours at pH 7.5 at 37°C.

Activators:

Ca²⁺ is required for enzyme activity.

Inhibitors:

The activity is inhibited by metal chelating agents such as EDTA, cysteine, o-phenanthroline and α₂-macroglobulin, a large plasma protein.

The most commonly used collagenase for tissue dissociation is a crude preparation from the extracellular culture filtrate of *Clostridium histolyticum*. It has collagenases as well as proteases which include clostripain, tryptic and a neutral protease activity. Crude collagenases are classified into different types based on different ratios of various proteolytic activities.

Type I: Original balance of collagenase, caseinase, clostripain and tryptic activities.

Type II: Prepared to contain higher clostripain activity.

Type IV: Prepared to contain lower tryptic activity levels to limit damage to membrane proteins and receptors with normal to high collagenase activity.

The difference in enzymatic ratios allow for selection of the collagenase type best suited for dissociation of a particular tissue. However crude collagenase do exhibit lot to lot variability and suitability of each lot of enzyme for disruption of a particular tissue should be determined empirically.

Type I: Suggested for epithelial, liver, lung and adrenal primary isolations.

Type II: Suggested for bone, heart, liver, thyroid and salivary primary cell isolation.

Type IV: Suggested for pancreatic islet primary isolation.

Directions :

1. Reconstitution:

Reconstitution can be done in any balanced salt solution. Dissolve lyophilized powder in a balanced salt solution and filter sterilize through 0.22 micron filter membrane. (Sterile filter with low protein binding properties like PES, PVDF should be used).

2. Dissociation using minced tissues:

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 - 100U/ml. Dissociation efficiency is increased if the digest is supplemented with 3 -5mM calcium chloride. Incubate at 37°C until disaggregation is complete.

Quality Control:

Appearance

White to dark brown powder.

Solubility

Clear colorless solution at 1mg in 1ml of water .

Assay

NLT 125U/mg

Caseinase Content

NLT 200U/mg

Clostripain Content

NMT 4.0U/mg

Tryptic Content

NMT 0.5U/mg

Cell Culture Test

Passes

Storage and Shelf Life:

Store Collagenase Type I powder at 2-8°C.

Reconstituted solution should be stored at -20°C.

Note: Avoid repeated freezing and thawing.

Shelf life of the product is 24 months.

Use before expiry date given on the product label.

Revision : 1 / 2012

Disclaimer :

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