

Collagenase Type IV

Cell Culture Tested

Product Code: TC214

Product Description :

Molecular weight: 68,000 to 125,000

Cas No: 9001-12-1

Enzyme Commission number: 3.4.24.3

Synonyms: Clostridiopeptidase A

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 at 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 collagenase is 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 - 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.

Quality Control:

Appearance

White to Dark brown powder.

Solubility

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

Assay

NLT 160U/mg

Caseinase Content

NLT 100U/mg

Clostripain Content

NMT 3U/mg

Tryptic content

NMT 0.1U/mg

Cell Culture Test

Passes

Storage and Shelf Life:

Collagenase Type IV, in the powdered form should be stored 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 :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.