Collagenase III

Cat: C8490
Size: 100mg/1g/5g
Storage: Store at -20℃ for 2 years.

Product Parameters:
CAS: 9001-12-1
English name: CollagenaseIII
Alias: Clostridiopeptidase A
Appearance (character): Brown freeze-dried powder
Enzyme activity/titer: 290u/mg
Solubility: 5mg/mL Water

Introduction:

Collagenase is a protease, an endopeptidase that specifically recognizes the Pro-X-Gly-Pro sequence(which occurs frequently in collagen but rarely in other proteins) and cleaves the peptide bond between the neutral amino acid(X) and glycine(Gly) of the sequence. Many proteases hydrolyze single-stranded denatured collagen polypeptides, but collagenase is the only protease that degrades natural collagen fibers with triple superhelical structure, which are widely found in connective tissue.

Collagenase is mainly divided into 4 types according to the difference of collagenase activity: collagenase type I, type II, type III and type IV, which is biased in application:

1) Type I Collagenase: contains relatively uniform activity of various enzymes (including collagenase, caseinase, clostridium protease, trypsin activity). Commonly used for the preparation of epithelial cells, liver, lung, fat and adrenal tissue cells;
2) Type II Collagenase: contains higher clostridium protease activity and is commonly used in the preparation of tissue-derived cells such as heart, bone, muscle, liver, thyroid, and cartilage;
3) Type III Collagenase: contains low protease activity and is commonly used in the preparation of breast cells;
4) Type IV Collagenase (Type IV Collagenase) : contains low pancreatic enzyme activity and is commonly used in preparation of islet cells or in cell preparation experiments where the integrity of the receptor needs to be maintained. This product is type III collagenase, commonly used in the preparation of breast cells and fetal cells.

Protocols(only for reference):

1. Preparation of collagenase storage solution

Add 1mL HBSS containing Ca^{2+} and Mg^{2+}(Hank's equilibrium salt solution containing Ca^{2+} and Mg^{2+}) to 100mg collagenase, gently vortex shock to fully dissolve it, and prepare 100mg/mL(that's 100×) storage solution. Then the bacteria were filtered with a low protein binding 0.22μm filter membrane, divided into small portions, and then frozen at -20℃ away from light.

Thaw on ice before use to avoid repeated freeze-thaw. The commonly used concentration for tissue and cell dispersion is 0.5-2.5mg/mL, and the commonly used concentration for cartilage digestion is 1-2mg/mL. The optimal working concentration needs to be determined according to specific experimental conditions or reference to the corresponding literature.

2. Separation of tissue

1) Use a sterile scalpel or scissors to cut the tissue into 3-4mm chunks;
2) The tissue blocks are wash several times with HBSS containing Ca^{2+} and Mg^{2+};
3) Add sufficient amount of HBSS containing Ca^{2+} and Mg^{2+} to immerse the tissue mass, and add collagenase to the required working concentration;
4) Incubate at 37℃ for 4-18h. The use of a horizontal shaker and the addition of 3mM $CaCl_2$ during digestion can improve digestion efficiency;
5) The dispersed cells can be screened using stainless steel or nylon mesh screen and collected for later use. The tissues that were not completely dissociated were further incubated with fresh collagenase working solution at 37℃.
6) The collected cells were washed with collagenase-free HBSS several times;
7) The cells are suspended in a cell culture solution and the live cell density is calculated using an automatic cell counter or other method;
8) The cells were inoculated on a cell culture dish using a suitable cell medium.

3. Organ perfusion

1) Adding collagenase to HBSS containing Ca^{2+} and Mg^{2+} preheated at 37℃, and adding 3mM $CaCl_2$ could improve the separation efficiency;
2) The corresponding organs were injected with collagenase working fluid at an optimized rate;
3) The perfusion liquid recovered in the above process is passed through the stainless steel or nylon mesh screen, so as to separate the dissociated cells or small fragments of tissue blocks from larger masses. The insufficiently dissociated tissues need to be further incubated at 37℃ with fresh collagenase working liquid;
4) The collected cells were washed with collagenase-free HBSS several times;
5) The cells are suspended in a cell culture solution and the live cell density is calculated using an automatic cell counter or other method;
6) The cells were inoculated on a cell culture dish using a suitable cell medium.

Notes:

1. If the biochemical reagents produced by our company are not specially marked, they are basically non-sterile packaging. If they are used in cell experiments, please pre-treat them in advance.
2. Once the solution is prepared, please store it separately to avoid product failure caused by repeated freezing and thawing.
3. Product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
4. This product is for scientific use only. Do not use for medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.
5. For your safety and health, please wear a laboratory coat, disposable gloves and a mask.