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Collagenase II

Product Number: C012156

Shipping and Storage

Transport at room temperature and store at 4℃.

Component

Component	C012156 - 100mg	C012156 -1g
Collagenase II	100mg	1g

Description

Collagenase, derived from Clostridium histolyticum, is a protease that can specifically recognize the Pro-X-Gly Pro sequence and cleave the peptide bond between the neutral amino acid (X) and glycine (Gly) in the sequence. This sequence appears frequently in collagen. Collagenase is the only protease that can degrade natural collagen fibers with triple helix that are widely present in connective tissue.

This product is type II collagenase, 2125U/mg solld, which can be used for the dissociation of tissues and cells such as heart, thyroid, salivary gland, liver, bone, cartilage, etc.

Definition of Activity

One enzyme activity unit refers to the amount of enzyme required to hydrolyze collagen to produce 1μmol L-leucine within 5 hours at 37 ° C and pH 7.5.

Protocol

1. Preparation of collagenase storage solution

Add 1mL of HBSS (Hank's balanced salt solution containing Ca^{2+} and Mg^{2+}) to each tube of 100 mg collagenase, gently vortex and shake to fully dissolve, and prepare a storage solution of 100 mg/mL (i.e. 100%). Filter and sterilize using a 0.22μm filter membrane with low protein binding, divide into small portions, and store in the dark at -20°C.

Thaw on ice before use to avoid repeated freezing and thawing. 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 required needs to be determined based on specific experimental conditions or reference to relevant literature.

2. Separation of Organizations

2.1. Cut the tissue into 3-4 mm tissue pieces using a sterile surgical knife or scissors;

2.2. Wash tissue blocks several times using HBSS containing Ca^{2+} and Mg^{2+};

2.3. Add sufficient HBSS containing $Ca^{2+}$and Mg^{2+} to immerse the tissue block, and add collagenase to the required working concentration;

2.4. Incubate at 37°C for 4-18 hours. Using a horizontal seeding bed and supplementing digestion with 3mM $CaCl_2$ can improve digestion efficiency;

2.5. Dispersed cells can be sieved using stainless steel or nylon mesh and collected for future use. Add an appropriate amount of fresh collagenase working solution to the partially dissociated tissue and continue incubating at 37°C;

2.6. Wash the collected cells several times using HBSS without collagenase;

2.7. Resuspend the above cells in cell culture medium and calculate the density of live cells using an automatic cell counter or other methods;

2.8. Inoculate cells onto a cell culture dish using appropriate cell culture medium.

3. organ perfusion

3.1. Adding collagenase and 3mM $CaCl_2$ to HBSS containing Ca^{2+} and Mg^{2+} preheated at 37°C can help improve separation

efficiency;

3.2. Inject collagenase working fluid into the corresponding organs at the optimized rate;

3.3. The recovered infusion solution from the above process is passed through a stainless steel or nylon mesh sieve to separate dissociated cells or small tissue fragments from larger clumps. Unfinished and dissociated tissues need to be further incubated with fresh collagenase working solution at 37°C;

3.4. Wash the collected cells several times using HBSS without collagenase;

3.5. Resuspend the above cells in cell culture medium and calculate the density of live cells using an automatic cell counter or other methods;

3.6. Inoculate cells onto a cell culture dish using appropriate cell culture medium.