Collagenase I from Clostridium histolyticum

A1492718

Storage： 2-8°C.

Introduction:

Collagenase is derived from Clostridium histolyticum and is a type of protease. It can specifically recognize the Pro-X-Gly-Pro sequence and cleave the peptide bond between the neutral amino acid (X) and glycine (Gly) in this sequence, and this sequence appears very frequently in collagen. Collagenase is the only protease that can degrade natural collagen fibers with a triple-helix structure, which are widely present in connective tissues. This product is Collagenase Type I with an enzyme activity of ⩾125 U/mg solid, and it can be used for the dissociation of tissues and cells such as epithelium, liver, lung, fat, and adrenal gland.

Usage method:

1. Preparation of Collagenase Stock Solution

1.1 Add 1 ml of HBSS (Hank's Balanced Salt Solution) containing Ca^{2+} and Mg^{2+} to each tube of 100 mg collagenase, gently vortex to dissolve it fully, and prepare a stock solution with a concentration of 100 mg/mL (i.e., 100×). Filter and sterilize using a 0.22 μm filter membrane with low protein binding property, aliquot into small portions, and store at -20℃ in the dark. Thaw on ice before use, and avoid repeated freezing and thawing. Common concentrations: the common concentration for tissue and cell dispersion is 0.5~2.5 mg/mL, and the common concentration for cartilage digestion is 1~2 mg/mL; the optimal working concentration should be determined according to specific experimental conditions or by referring to relevant literature.

2. Tissue Separation

2.1 Cut the tissue into tissue blocks of 3~4 mm in size using a sterile scalpel or scissors;

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

2.3 Add a sufficient amount of HBSS containing Ca^{2+} and Mg^{2+} to submerge the tissue blocks, and add collagenase to the required working concentration;

2.4 Incubate at 37℃ for 4~18 hours; using a horizontal shaker during digestion and supplementing digestion with 3 mM $CaCl_2$ can improve digestion efficiency;

2.5 Sieve the dispersed cells using a stainless steel or nylon mesh sieve, collect and set aside; for tissues that are not completely dissociated, add an appropriate amount of fresh collagenase working solution and continue incubation at 37℃;

2.6 Wash the collected cells several times with HBSS without collagenase;

2.7 Resuspend the above cells with cell culture medium, and calculate the viable cell density using an automatic cell counter or other methods;

2.8 Inoculate the cells on a cell culture dish using a suitable cell culture medium.

3. Organ Perfusion

3.1 Add collagenase to HBSS containing Ca^{2+} and Mg^{2+} preheated to 37℃; additionally adding 3 mM $CaCl_2$ helps improve separation efficiency;

3.2 Perfuse the corresponding organ with the collagenase working solution at an optimized rate;

3.3 Pass the perfusion solution recovered in the above process through a stainless steel or nylon mesh sieve to separate the dissociated cells or small tissue fragments from larger clumps; for tissues that are not fully dissociated, further incubate at 37℃ with fresh collagenase working solution;

3.4 Wash the collected cells several times with HBSS without collagenase;

3.5 Resuspend the above cells with cell culture medium, and calculate the viable cell density using an automatic cell counter or other methods;

3.6 Inoculate the cells on a cell culture dish using a suitable cell culture medium.