

Product Data Sheet

Product Name: Collagenase (Type D, animal free)

Cat. No.: GC27508

Chemical Properties

Cas. No. 9001-12-1

Formula

M.Wt

Solubility ≥ 50 mg/mL in H₂O

Storage Store at 4°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure

Collagenase (Type D, animal free)

Protocol

Storage Solution Preparation

1. Add 1 mL of HBSS (Hank's Balanced Salt Solution, containing Ca²⁺ and Mg²⁺) to 100 mg of collagenase, and shake to dissolve completely, preparing a 100 mg/mL (i.e., 100×) stock solution;
2. Filter-sterilize using a low-protein binding 0.22 µm filter membrane. Use immediately or aliquot into small portions and store at -20°C to -5°C in the dark;

Caution: Product has not been fully validated for medical applications. For research use only.

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3. Thaw on ice before use, avoiding repeated freezing and thawing. The commonly used concentration for tissue and cell dispersion is 0.5-2.5 mg/mL, and the commonly used concentration for cartilage digestion is 1-2 mg/mL. The optimal working concentration should be determined based on specific experimental conditions or by referring to relevant literature.

Tissue Dissociation

1. Cut the tissue into 3-4 mm pieces using a sterile scalpel or scissors;
 2. Wash the tissue pieces several times with HBSS containing Ca^{2+} and Mg^{2+} ;
 3. Add sufficient HBSS containing Ca^{2+} and Mg^{2+} to immerse the tissue pieces, and add collagenase to the required working concentration;
 4. Incubate at 37°C for 4-18 hours. Using a horizontal shaker and supplementing the digestion with 3 mM CaCl_2 can improve digestion efficiency;
 5. The dispersed cells can be collected using a stainless steel or nylon mesh sieve. For incompletely dissociated tissue, add an appropriate amount of fresh collagenase working solution and continue incubation at 37°C for further digestion;
 6. Wash the collected cells several times with HBSS without collagenase;
 7. Resuspend the cells in cell culture medium and calculate the viable cell density using an automated cell counter or other methods;
 8. Seed the cells in cell culture dishes using appropriate cell culture medium.
- ### Organ Perfusion
1. Add collagenase to HBSS containing Ca^{2+} and Mg^{2+} , preheated to 37°C. Adding 3 mM CaCl_2 helps improve separation efficiency;
 2. Perfuse the corresponding organ with the collagenase working solution at an optimized rate;
 3. Pass the perfusate collected during the above process through a stainless steel or nylon mesh sieve to separate the dissociated cells or small tissue fragments from larger clumps. Tissue that is not sufficiently dissociated needs to be further incubated with fresh collagenase working solution at 37°C.
 4. Follow steps 6-8 of the tissue separation protocol.

Background

Collagenase (Type D, animal free) does not contain animal-related components, and has collagenase and secondary protease activities similar to type 1 and type 2 collagenase. The activity of Collagenase (Type D, animal free) is higher than that of Collagenase (Type A, animal free).

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