Collagenase, Type I

Cat. No.: HY-E70005A

CAS No.: 9001-12-1

Target: MMP

Pathway: Metabolic Enzyme/Protease

Storage: Please store the product under the recommended conditions in the Certificate of Analysis.

Collagenase, Type I

SOLVENT & SOLUBILITY

In Vitro	H_2O : ≥ 50 mg/mL * "≥" means soluble, but saturation unknown.

BIOLOGICAL ACTIVITY

Description	Collagenase, Type I is a microbially derived matrix metalloproteinases (MMPs) and zinc peptidase. Collagenase, Type I breaks down collagens 1, 3, 7, 8, 10, gelatin, proteoglycans, aggrecan[1].
In Vitro	Type I collagenase is recommended for epithelial, liver, lung, and adrenal primary cell isolation. Preparation of storage solution 1. Add 1 mL Hank's Balanced Salt Solution (HBSS) with calcium and magnesium directly to 1 g vial of Collagenase.Vortex gently to ensure complete dissolution, and prepare a stock solution of 100 mg/mL (100X stock solution). 2. Filter sterilize 100X stock solution using a 0.22 μm filter with a low protein binding filtration unit. Use immediately or dispense into aliquots and store at -20°C to -5°C protected from light. 3. Thaw on ice prior to use. Commonly used concentrations for tissue and cell dispersion are 0.5-2.5 mg/mL and for cartilage digestion are 1-2 mg/mL, but the optimal working concentration required needs to be determined based on specific experimental conditions or by referring to the appropriate literature. Dissociate Tissue 1. Mince tissue into 3-4 mm pieces with a sterile scalpel or scissors. 2. Wash the tissue pieces several times with HBSS containing calcium and magnesium. 3. Add sufficient HBSS with calcium and magnesium to submerge tissue. Add collagenase to required working concentration. 4. Incubate at 37°C for 4-18 hours. Increased efficiency is obtained using a rocker platform and supplementing the digest with 3 mM $CaCl_2$. 5. Disperse cells by passing through a sterile stainless steel or nylon mesh. Remaining tissue fragments may be disaggregated by addition to fresh collagenase solution and further incubation at 37°C. 6. Wash dispersed cells several times by centrifugation in HBSS w/o collagenase. 7. Resuspend cell pellet, after the final wash step, in culture medium. Determine viable cell density using a Automated Cell Counter (alternate automated or manual methods may be used).

8. Seed cells into culture vessels containing appropriate media.

Organ Perfusion

1. Add collagenase to prewarmed (37°C) HBSS with calcium and magnesium. Addition of 3 mM $CaCl_2$ increases the efficiency of dissociation.
2. Perfuse organ at preoptimized rate for the particular organ.
3. Dispersed cells and tissue fragments are separated from larger pieces by passing the perfusate through a sterile stainless steel or nylon mesh. Remaining tissue fragments may be disaggregated by addition to fresh collagenase solution and further incubation at 37°C.
4. The steps are the same as for tissue isolation 6-8.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Hamzeh Alipour, et al. Therapeutic applications of collagenase (metalloproteases): A review. Asian Pac J Trop Biomed, 2016, 6(11): 975-981.

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