Collagenase Type IV
For research use only

Catalogue number: BI-1604

Product Description

Collagenase (from Clostridium histolyticum) is a protease with a specificity for the bond between a neutral amino acid (x) and glycine in the sequence Pro-X-Glyc-Pro. This sequence is frequently repeated in collagen and is unique among proteases in its ability to degrade the triple helical native collagen fibrils commonly found in connective tissue. The crude collagenase usually contains clostridiopeptidase A and a number of other proteases along with polysaccharidases and lipases and is commonly used for tissue dissociation. This crude enzyme is ideally suited for tissue dissociation since in addition to the enzyme required to attack native collagen and reticular fibers, it contains enzymes which hydrolyze the other proteins, polysaccharides, and lipids present in the extracellular matrix of connective and epithelial tissues.

Potency

One unit of the enzyme liberates 1 µM of L-leucine from collagen in 5 hours at +37°C, pH 7.5. Collagenase Type IV product's activity is guaranteed to be greater than 160 units/mg.

Notes

- Store at 2 to 8°C (-5 to -20°C after reconstitution)
- Avoid moisture and exposure to light.
- Avoid inhalation and skin contact.
- For research use only and not intended for human or animal diagnostic or therapeutic uses.
- Selected because of low tryptic activity.
- It is well-suited for digestion of pancreas islet cells, Mammary IV Islet (insulin receptor sites).

How to Use

1. Preparing stock and working solution:
   Dissolve the non-sterile, lyophilized enzyme in HBSS (with calcium and magnesium) OR PBS (with calcium and magnesium) Or DMEM/F12. Filter sterilize the solution with a cell culture approved filtration unit. Crude collagenase is most often used in concentrations from 0.1 to 0.5% (W/V) or 50 to 100 U/mL. Once reconstituted use immediately or store frozen. Thaw in the refrigerator immediately prior to use.
2. Dissociation of tissue

- The tissue is minced with a sterile scalpel or scissors.
- Wash the tissue several times in HBSS.
- The tissue fragments are soaked at +37°C. Increased efficiency is obtained using a rocker platform and supplementing the digest with 3 mM CaCl2.

3. Organ perfusion

- The digest is prewarmed to +37°C and perfused at a rate preoptimized for the particular organ. Addition of 3 mM CaCl2 increases the efficiency of dissociation.
- Dispersed cells and tissue fragments are separated from larger pieces by passing the mixture through a sterile stain less steel or nylon mesh. Fresh collagenase solution can be added to the fragments if further disaggregation is required.
- Wash several times to eliminate debris and enzyme solution. A density separation step (Nycodenz) will give a cleaner suspension.
- Resuspend the pellet in the culture medium and incubate under predetermined conditions.

Inhibitors

Metal chelating agents such as cysteine, EDTA or active oxygen species.

Citations

Rajaei, Bahareh, et al. "Pancreatic Endoderm Derived From Diabetic Patient Specific Induced Pluripotent Stem Cell Generates Glucose Responsive Insulin□Secreting Cells." Journal of cellular physiology 232.10 (2017): 2616-2625.