



Collagenase Type I

For research use only

Catalogue number: BI-1603

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 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 μ M of L-leucine from collagen in 5 hours at 37°C, pH 7.5. The product's activity is guaranteed to be greater than 125 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.
- Compared to other collagenase preparations, collagenase I has average levels of collagenase, caseinase, clostripain and tryptic activities and is well-suited for digestion of fat, adrenal, liver, and epithelial tissues.
- Metal chelating agents such as cysteine, EDTA or active oxygen species may inhibit the activity.

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 stainless 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.