Collagenase, 50 mg

from *Clostridium histolyticum*

Catalog Number **5030**

DESCRIPTION

Collagenases are enzymes that break down the native collagen that holds animal tissues together. Collagenase consists of a blend of enzymatic activities including collagenase, caseinase, clostripain and trypsin designed to effectively hydrolyze collagen and dissociate tissues. These enzymes are made by a variety of microorganisms and by many different animal cells. The most potent collagenase is the collagenase secreted by the anaerobic bacteria *Clostridium histolyticum*.

Bacterial collagenase is a crude complex containing a collagenase more accurately referred to as clostridiopeptidase A, which is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most frequently a neutral amino acid. Such sequences are often found in collagen, but only rarely in other proteins. While many proteases can hydrolyze single-stranded, denatured collagen polypeptides, clostridiopeptidase A is unique among proteases in its ability to attack and degrade the triple-helical native collagen fibrils commonly found in connective tissue.

True collagenase may cleave simultaneously across all three chains or attack at a single strand. Mammalian collagenases split collagen in its native triple-helical conformation at a specific site yielding fragments, TC A and TC B, representing 3/4 and 1/4 lengths of the tropocollagen molecule. After fragmentation the pieces tend to uncoil into random polypeptides and are more susceptible to attack by other proteases.

APPLICATIONS

Collagenase is provided as a dissociation reagent for collagen gels. Various collagenase products have been evaluated specifically for use with Advanced BioMatrix' s collagen products. This product is offered to improve the viability and functionality of isolated cells from collagen gels.

Collagenase is provided as a lyophilized, sterile powder in a 50 mg package size. After reconstitution, the product is ready-to-use.

Collagenase is not for human use as supplied.

CHARACTERIZATION

Source: From *Clostridium histolyticum*

Form: Lyophilized powder

Enzymatic Activity: Unit Dry Weight $\geq$ 125 U/mg
Caseinase $\geq$200 U/mg
Clostripain $\leq$ 4 U/mg
Tryptic $\leq$0.5 U/mg

Collagenase Unit Definition: One Unit liberates one micromole of L-leucine equivalents form collagen in 5 hours at 37°C, pH 7.5

Package Size: 50 mg

Sterilization: 0.22 micron filtered

Sterility: No growth

Storage: It is recommended that Collagenase lyophilized powder be stored at 2-10 °C. After reconstitution, the product is stable at 2 to 10 °C for up to 5 days.

INSTRUCTIONS FOR USE:

Use these recommendations as guidelines to determine the optimal gel dissociation procedure for your culture system.

1. Reconstitute the lyophilized collagenase powder (50 mg) in phosphate buffered saline or other pH neutral medium. A typical concentration of 1 mg/ml may be used.
2. Aspirate the culture medium from the collagen gel.
3. Wash the collagen gel 2X with pre-warmed PBS or other pH neutral medium to remove culture medium which could inhibit the degradation of collagen.
4. Add appropriate amount of collagenase solution (one gel volume should be sufficient).
   For example: For 24 well plates, containing 0.5 ml of gel, add 0.5 ml of collagenase solution.
5. Transfer to a 37ºC incubator for 30-60 minutes. To facilitate the faster dissociation of the collagen gel, the gel and collagenase solution can be pipetted up and down using a large bore pipette tip.
6. Incubate additional 30-60 minutes at 37°C, if necessary, to complete the digestion of the gel.

If required, pipette the gel/cell mixture every 15 minutes.
Note: Thicker gels or gels containing higher concentration of cells may require more time.

7. Once the gel is fully digested, add an equal volume of complete culture medium to the gel/cell mixture and then collect the cells. Rinse the culture vessel collecting any residual cells.
8. Centrifuge the cell suspension at 250 x g for 5 minutes at room temperature
9. Carefully aspirate the supernatant, and resuspend the cell pellet in 0.5 -1 ml of fresh medium,
10. Determine cell count and viability using a hemocytometer and Trypan Blue.