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DATASHEET

Collagenase IV

Catalogue No.:abx082456

Collagenase IV is an enzyme purified from Clostridium histolyticum intended for cell and tissue disaggregation of islet tissues/cells (insulin receptor sites).

Collagenase is a protease with specificity for the bond between a neutral amino acid (X) and glycine in the sequence Pro-X-Gly-Pro. This sequence is repeated frequently in collagen. Collagenase is unique among proteases in its ability to degrade the triplehelical native collagen fibrils commonly found in connective tissue. The collagenase most commonly used for tissue dissociation is a crude preparation containing clostripiopeptidase A and a number of other proteases, polysaccharidases, and lipases. This crude preparation is ideally suited for tissue dissociation because it contains the enzyme required to attack native collagen and reticular fibres, in addition to the enzymes which hydrolyze the other proteins, polysaccharides, and lipids in the extracellular matrix of connective and epithelial tissues. Crude collagenase can exhibit lot-to-lot variability and may produce occasional toxicity. The activity of these crude collagenase preparations has been correlated with their effectiveness at dissociating specific tissue types leading to the classification of crude collagenase preparations by type.

Target:	Collagenase IV
Origin:	Bacteria
Form:	Lyophilized
Reconstitution:	1. Add 1 ml Hank's Balanced Salt Solution (HBSS) with calcium and magnesium directly to 1 g of Collagenase. Vortex gently to ensure complete dissolution. 2. Transfer the solution to a clean tube. 3. Determine volume of HBSS with calcium and magnesium required to bring the collagenase solution to 100 U/µl (1000X stock solution). Rinse the vial with this volume of HBSS with calcium and magnesium, and combine. 4. Filter the 1000X stock solution with a low protein binding filtration unit. Use immediately or proceed to the next step. 5. Dispense into aliquots and store at -20°C to -5°C in the dark. 6. Thaw on ice prior to use. Avoid multiple freeze/thaw cycles. We recommend using collagenase at 50–200 U/ml concentration (or 0.1–0.5% W/V).
Conjugation:	Unconjugated
CAS Number:	9001-12-1
Validity:	The validity for this kit is 6 months.
Storage:	Store at 2°C to 8°C upon receipt. After reconstitution, store at -20°C to -5°C in the dark.
Biological Activity:	≥125 CDU/mg (CDU = collagen digestion units) One protease unit liberates 1 µmol of L-leucine equivalents from collagen in 5 hours at 37°C, pH 7.5.

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Sample Type: Islet tissues/cells (insulin receptor sites).

Directions for use: **Dissociate tissue**

1. Ground the 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 50–200 U/ml.
4. Incubate at 37°C for 4–18 hours. Increased efficiency can be obtained by 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. The 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 the cell pellet, after the final wash step, in culture medium. Determine viable cell density using an automated cell counter or other automated or manual method.
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 preoptimised rate for the particular organ.
3. Separate dispersed cells and tissue fragments from larger pieces by passing the perfusate through a sterile stainless steel or nylon mesh.
The remaining tissue fragments may be disaggregated by addition to fresh collagenase solution and further incubation at 37°C.
4. Wash dispersed cells several times by centrifugation in HBSS w/o collagenase.
5. Resuspend the cell pellet, after the final wash step, in culture medium. Determine viable cell density using an automated cell counter or other automated or manual method.
6. Seed cells into culture vessels containing appropriate media.

Note: This product is for research use only.