

I型胶原酶

Collagenase, Type I, powder



Catalog Number : MG1241

CAS : 9001-12-1

Packaging Size : 100mg ☐ 1g ☐

Technical literature is available at: www.mesgenbio.com.

E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com

产品描述

I型胶原酶是从溶组织梭菌制备，用于细胞和组织的解离。胶原酶是一种蛋白酶。I型胶原酶可用于上皮、肝、肺、脂肪和肾上腺等组织和细胞的解离。

Description

Collagenase is a protease that cleaves the bond between a neutral amino acid (X) and glycine in the sequence Pro-X-Gly-Pro, which is found with high frequency in collagen. Collagenase is unique among proteases in its ability to degrade the triple-helical native collagen fibrils commonly found in connective tissues such as skin, tendon, blood vessels, and bone. Collagenase disaggregation is suitable for the culture of human tumors, mouse kidney, human adult and fetal brain, and many other tissues including epithelium. Collagenase is relatively gentle, dissociates well at physiological temperature and pH, and requires no mechanical agitation or special equipment.

Collagenase Type I is isolated from *Clostridium histolyticum* and packaged as a lyophilized, non-sterile powder for research use in cell or tissue dissociation, and organ perfusions. Collagenase Type I activity is guaranteed to be greater than 125 units/mg. Compared to other collagenase preparations, Collagenase Type I has average levels of collagenase, caseinase, clostripain, and tryptic activities, and is well-suited for the digestion of fat, adrenal, and liver cells or tissues.

Reconstitute Collagenase

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.

Do not eat Store at -20° C

2. Transfer to a clean tube.
3. Determine volume of HBSS with calcium and magnesium required to bring collagenase solution to 100 U/μL (1000X stock solution). Rinse vial with this volume of HBSS with calcium and magnesium, and combine.
4. Filter sterilize 1000X stock solution with a low protein binding filtration unit. Use immediately or proceed to step 5.
5. Dispense into aliquots and store at -20°C to -5°C protected from light.
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).

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 50–200 U/mL.
4. Incubate at 37°C for 4–18 hours. Increased efficiency is obtained using a rocker platform and supplementing the digest with 3 mM CaCl₂.
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.
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 CaCl₂ 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. Wash dispersed cells several times by centrifugation in HBSS w/o collagenase.
5. Resuspend cell pellet, after the final wash step, in culture medium.
6. Seed cells into culture vessels containing appropriate media.

Unit Definition

One protease unit liberates 1 μ mol of L-leucine equivalents from collagen in 5 hours at 37°C, pH 7.5.

Storage condition

-20°C

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For Research Use Only. Not For Use In Diagnostic Procedures.