For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

IV型胶原酶

Collagenase, Type IV, powder

Catalog Number: MG1243

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

IV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。IV型胶原酶含有低胰酶活性,通常用于胰岛细胞的制备,或者需要维持受体完整性的细胞制备实验。

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 IV is isolated from Clostridium histolyticum and packaged as a lyophilized, non-sterile powder for research use in

packaged as a lyophilized, non-sterile powder for research use in cell or tissue dissociation and organ perfusions. Collagenase Type IV activity is guaranteed to be greater than 160 units/mg. Compared to other collagenase preparations, Collagenase Type IV has low tryptic activity and is well-suited for the digestion of islet cells from the pancreas.

Reconstitute Collagenase

- 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.
- 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

Store at -20° C

magnesium, and combine.
4. Filter sterilize 1000X stock solution with a low protein binding

solution). Rinse vial with this volume of HBSS with calcium and

5. Dispense into aliquots and store at $-20\,^{\circ}\text{C}$ to $-5\,^{\circ}\text{C}$ protected from light.

filtration unit. Use immediately or proceed to step 5.

 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

Do not eat

- 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.
- 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.
- 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.
- Resuspend cell pellet, after the final wash step, in culture medium.
- 8. Seed cells into culture vessels containing appropriate media.

Organ Perfusion

- 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.
- Dispersed cells and tissue fragments are separated from larger pieces by passing the perfusate through a sterile stainless steel

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- 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 $\mu mol\ of\ L\text{-leucine}$ equivalents from collagen in 5 hours at 37 °C, pH 7.5.

Storage condition

-20°C

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