Collagenase II

REF: EG25306-S/M

Storage Condition

Transport at room temperature and store at 4℃ protected from light.

Components

Component	EG25306S	EG25306M
Collagenase II	100 mg	1 g

Description

Collagenase products are purified from *Clostridium histolyticum*, which is a protease with specificity for the bond between a neutral amino acid (X) and glycine in the sequence Pro-XGly-Pro. This sequence is found in high frequency in collagen. Collagenase is unique among proteases in its ability to degrade the triplehelical native collagen fibrils commonly found in connective tissue.

This product is Type II collagenase with ≥125 U/mg solid, suitable for dissociation of tissues and cells in heart, thyroid, salivary gland, liver, bone, cartilage, etc.

Definition of Activity Unit

One unit liberates one micromole of L-leucine equivalents from collagen in 5 hours at 37℃ , pH7.5.

Protocol

1. Preparation of Collagenase Storage Solution

Add 1 ml corresponding buffer solution (determined according to specific experimental conditions or by referring to relevant literature), such as Hank's Balanced Salt Solution (HBSS) with calcium and magnesium, directly to 100 mg vial of Collagenase, gently vortex to ensure complete dissolution, prepare as a stock solution at 100 mg/ml (i.e., 100×). Sterilize by filtration through a low protein binding 0.22 μm membrane filter. Dispense into aliquots and store at -20 ℃ protected from light.

Thaw on ice prior to use. Avoid multiple freeze/thaw cycles. We recommend using collagenase at 0.5~2.5 mg/ml concentration for tissue and cell dispersion, 1~2 mg/ml concentration for cartilage digestion. Optimal concentration should be determined based on specific experimental conditions or by referring to relevant literature.

2. Dissociate Tissue

(1) Mince tissue into 3~4 mm pieces with a sterile scalpel or scissors.

(2) Wash the tissue pieces several times with corresponding buffer solution (such as HBSS).

(3) Add sufficient buffer solution to submerge tissue. Add collagenase to the desired concentration.

(4) Incubate at 37℃ for 4~18 hours. Increased efficiency is obtained 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. Remaining tissue fragments may be disaggregated by addition to fresh collagenase solution and further incubation at 37℃ .

(6) Wash dispersed cells several times by centrifugation in buffer solution w/o collagenase.

(7) Resuspend cell pellet, after the final wash step, in culture medium. Determine viable cell density using an automated cell counter or other methods.

(8) Seed cells into culture vessels containing appropriate media.

3. Organ Perfusion

(1) Add collagenase to prewarmed (37 ℃) corresponding buffer solution (such as HBSS). Addition of 3 mM $CaCl_2$ 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℃ .

(4) Wash dispersed cells several times by centrifugation in buffer solution w/o collagenase.

(5) Resuspend cell pellet, after the final wash step, in culture medium. Determine viable cell density using an automated cell counter or other methods.

(6) Seed cells into culture vessels containing appropriate media.

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