gibco by life technologies™

Collagenase

Description

Life Technologies' group of collagenase products are purified from *Clostridium histolyticum*. They are intended for cell and tissue disaggregation. 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 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. 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 fibers, in addition to the enzymes which hydrolyze the other proteins, polysaccharides, and lipids in the extracellular matrix of connective and epithelial tissues. Crude collagenase does 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. These selected types have been found to give better performance in preparation of cells from the various tissues (Table 1).

Product	Catalog No.	Amount	Storage
Collagenase:			
Type I	17100-017	1 g	2°C to 8°C
Type II	17101-015	1 g	2°C to 8°C
Type IV	17104-019	1 g	2°C to 8°C
Collagenase, lyophilized	17018-029	500 mg	2°C to 8°C

Product Use

For Research Use Only. Not for use in diagnostic procedures

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Avoid inhalation and skin contact.

Unit Definition

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

Table 1: Product Selection

Collagenase	Tissue / Cell type
Type I	Epithelial, Adrenal, Lung, Fat
Type II	Heart, Thyroid, Salivary, Liver, Bone, Cartilage
Type IV	Islet (insulin receptor sites)

Use

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.
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 $CaCl_2$.
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°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. Determine viable cell density using a Countess® Automated Cell Counter (alternate automated or manual methods may be used).
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 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. Determine viable cell density using a Countess® Automated Cell Counter (alternate automated or manual methods may be used).
6. Seed cells into culture vessels containing appropriate media.

Related Products

Product	Catalog No.
HBSS, calcium, magnesium, no phenol red	14025
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

		
Manufacturer	Catalog number	Batch code
		
Caution, consult accompanying documents	Consult instructions for use	Temperature Limitation

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**