Collagenase Type I Solution 0.25% in PBS with 20% FBS

CA091

Storage

Store at -20 °C.
Product is stable at -20 °C until expiration date on label.
Storage at 2 - 8 °C is not recommended.
This solution should be thawed, aliquoted into working volumes, and refrozen.
Avoid repeated freeze-thaw cycles.

Contents

- Product manual
- Collagenase Type I 0.25% in PBS with 20% FBS

Introduction

This product has been optimized for solubilization of collagen gels and the efficient disruption of embryoid bodies (EBs) derived from embryonic stem (ES) cells.
Collagenase is purified from Clostridium histolyticum and is assayed at 200-300 units per mg. One unit liberates peptides from collagen equivalent in ninhydrin color to 1.0 micromole of leucine in 5 hrs at pH 7.4 at 37°C in the presence of calcium ions.

Instructions for use

1. Thaw collagenase at room temperature.
2. Harvest EBs and wash in Iscove's MDM with 2% fetal bovine serum (FBS).
3. Remove supernatant carefully as to not disturb the loose pellet.
4. Add 2 - 3 mL Collagenase and incubate at 37 °C for one hour.
5. Disrupt EBs by passaging media and cells through a 21G, 1" needle on a 3cc syringe (3 times).
6. Add Iscove's MDM with 5% FBS to neutralize Collagenase and pellet cells by centrifugation at 300 x g for 5 - 8 minutes.
7. Resuspend the cells in a minimum volume of Iscove's MDM with 2% FBS.

Solubilization of thin collagen types I gels: This product can be used for the solubilization of thin collagen type 1 gels.

1. Thaw collagenase solution at room temperature.
2. For a 1 - 1.5 mL collagen thin gel, add 1 mL of collagenase solution.
3. Incubate at 37 °C for a 15 - 60 minutes. The optimal time will vary depending on the density of the collagen gel and the cell types being isolated.

Note: The appropriate culture medium is dependent on the cell type being isolated and the downstream application. For example, if the isolated cells will be set up in culture, use the same culture medium to wash the cells. If the isolated cells will be ued for applications including RNS or DNA isolation, a medium such as PBS may be suitable.

4. Gently pipette solution into a 10X volume of culture medium.
5. Centrifuge at 300 x g for 7 - 10 minutes to recover cells. Wash cells with culture medium twice prior to use, to ensure Collagenase and collagen are effectively removed.

Related Product

Product Name	Cat No
Scraptase	CA110
Trypsin-EDTA (10X), 0.5%	CA015
Trypsin, 2.5% (10X)	CA019
Trypsin-EDTA (1X), 0.05%	CA020

GenDEPOT PO Box 454 Barker, Tx 77413 tech@gendepot.com