Collagenase/Hyaluronidase (10X)

CA094

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, aliqouted into working volumes and refrozen.
Avoid repeated freeze-thaw cycles.

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Introduction

10X collagenase/Hyaluronidase in Dulbecco's Modified Eagle's Medium (DMEM) for the enzymatic dissociation of human and mouse mammary tissue.

Instructions for use

Collagenase/Hyaluronidase enzyme soluiton is provided as a 10X stock solution, and should be diluted 10-fold as outlined in the protocols below.
Note: Avoid the use of glass pipettes and tubes when handling mammary epithelial cells. These cells will stick to the glass.

1.0 A Dissociation of Human Mammary Tissue

1. Transport human mammary tissue from the operation room on ice in sterile specimen cups in Complete Medium supplemented with 5% fetal bovine serum (FBS: #F0600).
2. Transfer tissue to sterile glass petri dishes, mince with scalpels and then transfer to tissue dissociation flasks. Glass petri dishes can be used for this initial dissociation, as the concentration of epithelial cells is very low.
3. Dilute 1 part 10X Collagenase/Hyaluronidase with 9 parts Complete Medium and add to the minced tissue in the dissociation flasks. Ensure that the tissue is well spended in the enzyme mixture and the final volume is level with the widest portion of the flask. Cover the opening of the flask with sterile aluminum foil.
4. Gently dissociate the minced tissue on a rotary shaker at 37 °C until all large tissue fragments are digested. Typical digestion time is 16 hours (overnight) for normal human mammary tissue. Longer digestion times may be required for tough fibrous tissue, shorter digestion times for softer tissue.
The flasks should be sealed if the rotary shaker is not in a 5% CO_2 incubator.
5. After dissociation, transfer the dissociated tissue to 50 mL centrifuge for 30 seconds at 80 x g.
6. Discard the overlying liquefied fat layer. The pellet ("A" pellet) is highly enriched for epithelial organoids. To generate a single cell suspension from the "A" pellet, refer to Section 2.0
7. Transfer the supernatant to a new 50 mL centrifuge tube and centrifuge at 200 x g for 3 minutes. The pellet ("B" pellet) from this second centrifugation contains variable numbers of epithelial cells, stromal cells and red blood cells. To generate a single cell suspension from the "B" pellet, refer to section 2.0.
8. The supernatant from the second centrifugation is enriched for human mammary fibroblasts. To collect, transfer the supernatant to a new 50 mL centrifuge tube and centrifuge at 350 x g for 5 minutes.
9. The different cell fractions can now be cryopreserved. It is recommended that cells are cryopreserved in Complete Medium supplemented with 50% FBS and 6% Dimethyl Sulfoxide.

1.0B Dissociation of Mouse Mammary Tissue

1. Dilute 1 part 10X Collagenase/Hyaluronidase with 9 parts Complete Medium supplemented with 5% FBS and place into a 14 mL or 50 mL centrifuge tube. Approximately 2 - 5 mL of the Medium/Collagenase/Hyaluronidase/FBS solution will be required for every 2 mammary glands to be dissociated.
2. Resect mammary glands and transfer to a sterile glass petri dish. Mince with scalpels in a cross-wise pattern until glands are rendered to a paste. Transfer the mammary tissue to the tube containing Medium/Collagenase/Hyaluronidase/FBS and incubate 6 - 8 hours at 37 °C with occasional pipetting and vortexing. Mouse mammary glands can be dissociated for shorter lengths of time (1-2 hours), however the mammary stem cell yield is dramatically (~80%) reduced (and in some cases, absent). A short tissue dissociation time results in a high yield of liberated lymphocytes and stromal cells, but the stem cells are apparently lost in the filtration stage (Section 2.0, Step 6), even following trypsin and dispase treatment. Conversely, digestion longer than 8 hours results in over-digestion of the cells and a decrease in stem cell yield (but not total cell yield).
It has been observed that mammary repopulating units (MRU) frequencies and yields are significatnly higher when the mammary glands are Collagenase/Hya -luronidase digested in growth factor-free and serum-free media such as DMEM/F12 when compared to media containing growth factors and serum. However, total viable mammary cell and colony-forming cell (CFC) yields are in the latter medium (J Stingl, unpublisheddata).
3. After dissociation, centrifuge the cells at 350 x g and discard the supernatant.
4. Resuspend the pellet with a 1:4 mixture of cold Hank's Balanced Salt Solution Modified supplement with 2% FBS and ammonium chloride (NH_4Cl) and centrifuge at 350 x g for 5 minutes. The resultant pellet contains epithelial cell organoids as well as stromal cells and lymphocytes. To generate a single cell suspension of mammary epithelial cells, refer to Section 2.0

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Storage

2.0 Generation of Single Cell Suspensions from Dissociated Human and Mouse Mammary Tissue

1. Add 1-5 mL of pre-warmed Trypsin-EDTA to the Collagenase/Hyaluronidase -dissociated mammary cells and resuspend cells. For human tissue, the best starting material is “A” pellets. “B” pellets may also be used, however the success of the cultures derived from these pellets is more variable due to the variable epithelial content. For mouse cells, use the entire mammary cell pellet.
2. Gently pipette up and down with a P1000 micropipettor for 1 - 3 minutes. The sample should become very stringy due to lysis of dead cells and the release of DNA.
3. Add 10 mL of cold Hank’s Balanced Salt Solution modified supplemented with 2% FBS and centrifuge at 350 x g for 5 minutes.
Hanks’ + FBS solution is now referred to as HF.
4. Remove as much of the supernatant as possible. The cells may be a large ‘stringy mass’ floating in the HF.
5. Add 2 mL of pre-warmed 5 mg/mL Dispase and 200 uL of 1 mg/mL DNase I. Pipette the sample for one minute with a P1000 micropipettor to further dissociate cell clumps. The sample should now be cloudy, but not stringy. If still stringy add more DNase I.
6. Dilute the cell suspension with an additional 10 mL of cold HF and filter the cell suspension through a 40 um cell strainer into a new 50 mL centrifuge tube. Centrifuge at 350 x g for 5 minutes and discard the supernatant.
7. If the cell pellet is heavily contaminated with red blood cells, resuspend the pellet in a 1:4 mixture of cold HF: ammonium chloride (NH_4Cl), centrifuge at 350 x g for 5 minutes and discard the supernatant.
8. Cells are now ready for use in desired application.

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

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