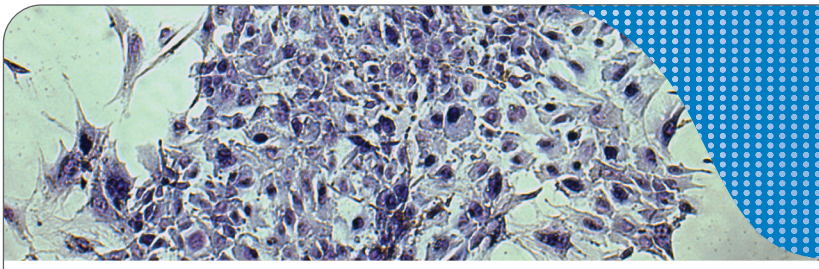

TECHNICAL BULLETIN

A GUIDE TO SOLID MAMMARY AND PROSTATE TISSUE DISSOCIATION

SOLID MAMMARY AND PROSTATE TISSUE DISSOCIATION

Many techniques such as cell separation, flow cytometry or stem cell assays are dependent on the cells being in a single cell suspension. Clumpy cells or partially dissociated tissues can lead to problems in assay performance or data analysis. Unfortunately, generating a single cell suspension from solid mammary or prostate tissues can be complicated and difficult. This Technical Bulletin outlines the collection of procedure for dissociating mammary and prostate tissue to a single cell suspension.

1.0 MAMMARY TISSUE

1.1.1 Dissociation of Human Mammary Tissue

Application: These procedures (1.1.1, 1.1.2 and 1.2) have been optimized to dissociate human or mouse mammary tissue to a single cell suspension for use in cell separation, flow cytometry or progenitor cell assays such as Ma-CFC (mammary colony-forming cell) or MRU (mammary repopulating unit) assays.

1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in DMEM/F12 (Catalog #36254) supplemented with 5% fetal bovine serum (FBS; Catalog #06100).
2. Transfer the tissue to sterile glass petri dishes, mince with a scalpel and then transfer to tissue dissociation flasks (Catalog #27300).
3. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) with 9 parts DMEM/F12 (Catalog #36254) supplemented with 2% bovine serum albumin (Fraction V), and add to the minced tissue in the dissociation flasks. Ensure that the tissue is well suspended 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 for ~16 hours or overnight (for normal human mammary tissue).
5. After dissociation, transfer the dissociated tissue to 50 mL centrifuge tubes, and centrifuge at 80 x *g* for 30 seconds with the brake on.
6. Discard the overlying liquefied fat layer and transfer the supernatant to another 50 mL tube.
7. The remaining pellet ("A" pellet) is highly enriched for epithelial organoids. To generate a single cell suspension from the "A" pellet, please refer to Section 1.2.
8. Centrifuge the supernatant at 200 x *g* for 3 minutes with the brake on and transfer the supernatant to a new 50 mL tube. 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, please refer to Section 1.2.
9. The supernatant from the second centrifugation is a single cell suspension enriched for human mammary fibroblasts. To collect these cells, centrifuge at 350 x *g* for 5 minutes with the brake on.
10. The different cell fractions can now be cryopreserved. It is recommended that cells are cryopreserved in Complete EpiCult®-B Medium (Catalog #05601) supplemented with 50% FBS (Catalog #06100) and 6% Dimethyl Sulfoxide.

1.1.2 Enzymatic Dissociation of Mouse Mammary Tissue

1. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) mixture with 9 parts Complete EpiCult®-B Medium (Mouse; Catalog #05610) supplemented with 5% FBS (Catalog #06100) and place into a 14 mL or 50 mL centrifuge tube. Approximately 2 - 5 mL of the EpiCult®-B 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 EpiCult®-B Medium/Collagenase/Hyaluronidase/FBS and incubate 6 - 8 hours at 37°C with occasional pipetting and vortexing.
3. After dissociation, centrifuge the cells at 350 x *g* for 5 minutes with the brake on and discard the supernatant.
4. Resuspend the pellet in a 4:1 mixture of ammonium chloride (NH_4Cl; Catalog #07800) and cold Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x *g* for 5 minutes with the brake on. Discard the supernatant. The pellet contains epithelial cell organoids as well as stromal cells and lymphocytes. To generate a single cell suspension of mammary epithelial cells, please refer to Section 1.2.

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

1. Add 1 - 5 mL of pre-warmed Trypsin-EDTA (Catalog #07901) to the Collagenase/Hyaluronidase-dissociated mammary cells (pellets from sections 1.1.1 and 1.1.2) and resuspend cells. For human tissue, the fraction most enriched for epithelial cells is the "A" pellet (Section 1.1.1, Step 7).
2. Gently pipette up and down with a P1000 disposable tip for 1 - 3 minutes.
3. Add 10 mL of cold Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x *g* for 5 minutes with the brake on. The Hanks' + FBS solution is now referred to as HF.
4. Remove as much of the supernatant as possible.
5. Add 2 mL of pre-warmed 5 mg/mL Dispase (Catalog #07913) and 200 µL of 1 mg/mL DNase I (Catalog #07900). Pipette the sample for 1 minute with a P1000 disposable plastic tip.

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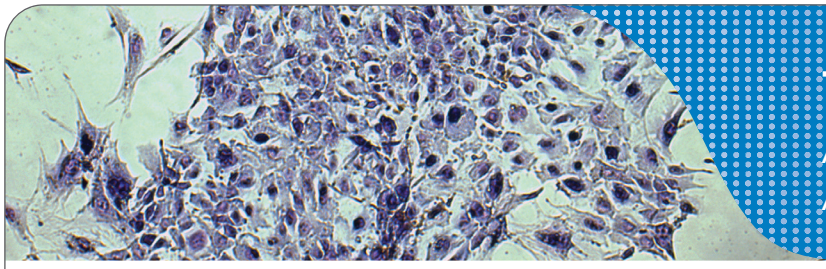

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6. Dilute the cell suspension with an additional 10 mL of cold HF and filter the cell suspension through a 40 µm cell strainer (Catalog #27305) into a new 50 mL centrifuge tube. Centrifuge at 350 x *g* for 5 minutes with the brake on and discard the supernatant. Resuspend cells in a medium suitable for subsequent assays.
7. Count viable cells using Trypan Blue (Catalog #07050) and a hemacytometer.

2.0 PROSTATE

2.1 Enzymatic Dissociation of Human and Mouse Prostate Tissue

Application: This procedure has been optimized to generate a single cell suspension for use in flow cytometry or progenitor cell assays.

1. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) mixture with 9 parts DMEM/F12 (Catalog #36254) supplemented with 5% FBS (Catalog #06100) and place into a 14 mL or 50 mL centrifuge tube. Approximately 2 - 5 mL of the DMEM/F12/Collagenase/Hyaluronidase/FBS solution will be required for every 2 - 3 mouse prostates. The volume of dissociation mix for human samples will be dependent on the size of the sample (typically 10 times more solution than the volume of the sample).
2. Resect prostates and transfer to a sterile petri dish containing cold PBS. Using a dissecting microscope, a fine set of forceps and scissors remove residual amounts of fat from prostate tissue.
3. Transfer the prostate tissue to the tube containing DMEM/F12/Collagenase/Hyaluronidase/FBS and incubate for 3 hours at 37°C.
4. After dissociation, centrifuge the cells at 350 x *g* for 5 minutes with the brake on and discard the supernatant.
5. Resuspend the pellet in 5 - 6 mL of 0.25% Trypsin-EDTA (Catalog #07901) and leave on ice for 1 hour.
6. Add 10 mL of cold Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x *g* for 5 minutes with the brake on.
7. Remove as much of the supernatant as possible.
8. Add 2 mL of pre-warmed 5 mg/mL Dispase (Catalog #07913) and 200 µL of 1 mg/mL DNase I (Catalog #07900). Pipette the sample for 1 minute with a P1000 disposable plastic tip.
9. Add 10 mL of cold Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and filter the cell suspension through a 40 µm cell strainer (Catalog #27305) into a new 50 mL centrifuge tube. Centrifuge at 350 x *g* for 5 minutes with the brake on and discard the supernatant. Resuspend cells in a medium suitable for subsequent assays.
10. Count viable cells using Trypan Blue (Catalog #07050) and a hemacytometer.

TABLE 1: Support Products

PRODUCT	QUANTITY	CATALOG #
Fetal Bovine Serum	100mL	06100
DMEM/F12	500mL	36254
Hanks' Balanced Salt Solution Modified	500mL	37150
10X Collagenase/ Hyaluronidase	10mL	07912
Trypsin-EDTA	500mL	07901
Dispase (5mg/ml)	100mL	07913
DNase I (1mg/ml)	1mL	07900
NH_4Cl	100mL	07800
Trypan Blue	100mL	07050
Tissue Dissociation Flask	1 each	27300
40 µm Cell Strainer	50 per case	27305

BACKGROUND REFERENCES FOR EPICULT-B®

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