4.0 SPLEEN

4.1 Enzymatic Dissociation of Mouse Spleen

**Application:** This procedure is optimized for the isolation of dendritic cells from mouse spleen. This procedure uses StemCell’s Spleen Dissociation Medium (Catalog #07915). For more information, please refer to the Product Information Sheet.

1. With tissue in a 60 mm dish, use dissection scissors and forceps to mince 1 – 2 freshly isolated mouse spleens into a homogenate paste. Spleen fragments should be less than 1 mm in size.
2. Resuspend the minced tissue in 4 mL of Spleen Dissociation Medium (Catalog #07915).
3. Transfer the solution into the tube temporarily containing Spleen Dissociation Medium.
4. Incubate the tube for 10 minutes at 37°C, preferably with constant agitation.
5. Gently pass the tissue several times through a 16-gauge blunt-end needle (Catalog #08110) using a 3 cc syringe.
6. Add EDTA to a final concentration of 10 mM (e.g. 80 µL of 0.5 M stock) and incubate horizontally with gentle rocking at room temperature for 5 minutes.
7. Prime a 70 µm filter by pouring 5 mL of FBS with 2% PBS (Catalog #07905) through the mesh. Transfer the entire suspension through the filter into a 50 mL conical screw-cap tube.
8. Rinse the empty Spleen Dissociation Medium tube and mesh filter with an additional 10 mL of FBS with 2% PBS.
9. Centrifuge the 50 mL conical screw-cap tube at 350 x g for 10 minutes with the brake on and discard the supernatant.
10. Resuspend the cells in FBS with 2% FBS and count viable cells using Trypan Blue (Catalog #07050) or nucleated cells with 3% Acetic Acid with Methylene Blue (Catalog #07060) and a hemacytometer.

4.2 Mechanical Dissociation of Mouse Spleen

**Application:** This procedure is optimized for the isolation of T and B lymphocytes from mouse spleen.

1. In a 100 mm dish, place 1 – 2 freshly isolated mouse spleens into 10 mL of desired medium such as PBS with 2% FBS (Catalog #07905).
2. Wet 2 frosted-end glass microscope slides with ice-cold medium.
3. Place the spleen on the frosted side of one slide and nick the capsule with the edge of the frosted side of the other slide. Crush the spleen between the slides by making them parallel.
4. Hold slides containing the spleen stationary and gently rotate the other slide to dissociate the spleen and free the cells.
5. Rinse slides with medium to recover remaining cells.
6. Prime a 70 µm filter by pouring 5 mL of PBS with 2% FBS (Catalog #07905) through the mesh. Transfer the entire suspension through the filter into a 50 mL conical screw-cap tube.
7. Centrifuge the 50 mL conical screw-cap tube at 350 x g for 10 minutes with the brake on and discard the supernatant.
8. Resuspend the cells in PBS with 2% PBS and count viable cells using Trypan Blue (Catalog #07050) or nucleated cells with 3% Acetic Acid with Methylene Blue (Catalog #07060) and a hemacytometer.

5.0 PROSTATE

5.1 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 #07915) mixture with 9 parts DMEM/F-12 (Catalog #36254) supplemented with 5% FBS (Catalog #06100) and place into a 14 mm or 50 mL centrifuge tube. Approximately 2 – 5 mL of the DMEM/F-12/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. Resuspend 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/F-12/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 Hank’s Balanced Salt Solution Modified (Catalog #07150) 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 Hank’s Balanced Salt Solution Modified (Catalog #07150) supplemented with 2% FBS (Catalog #06100) and filter the cell suspension through a 40 µm cell strainer (Catalog #27323) 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.

Summary of Dissociation Procedures

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Solid 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 tissues can be complicated and difficult. This Technical Bulletin is a collection of procedures that reproducibly process a variety of solid tissues to a single cell suspension.
1.5 BONE

1.1 Isolation of Cells from Mouse Bone

Application: This procedure has been optimized to isolate cells from mouse bone prior to mesenchymal cell enrichment, expansion or CFU-F assay.

1. Isolate and clean mouse femur and tibia separately.
2. Place clean bones in sterile mortar containing 10 mL PBS with 2% FBS (Catalog #07905) and 1 mM EDTA (now referred to as Buffer).
3. Crush bones gently with pestle, using only enough force to crack open the bones. Agitate gently to free bone marrow (BM) from bone fragments. Buffer containing BM can be removed and used for other applications.
4. Add 10 mL fresh Buffer and repeat agitation and removal of BM. Repeat wash step for a total of 6 washes or until the majority of the BM has been removed (bone fragments turn white in color).
5. Transfer the bone fragments to a 100 mm dish. Add 2 mL PBS and gently agitate to free bone marrow (BM) from bone fragments. Buffer containing BM can be removed and used for other applications.
6. Using a scalpel, chop the bone fragments into fine pieces (1-2 mm fragments).
7. Transfer the bone fragments and collagenase solution to a 50 mL polystyrene tube and add further 0.25% Collagenase Type I solution (Catalog #07907) to final volume of 2 mL per mouse, or a minimum of 10 mL.
8. Seal lid and place tube in a shaking 37°C waterbath at maximum speed for 45 minutes. If using a bacterial culture shaker, set speed to ~200 rpm.
9. After 45 minutes, add buffer to a final volume of 30 mL and agitate gently to settle, collect supernatant and filter through a 70 µm cell strainer. Mix bone fragments with an additional 10 mL of Buffer and agitate fragments to settle for 3-4 minutes. Filter the wash through the 70 µm strainer, combining with the previously collected cells.
10. Centrifuge at 350 x g for 10 minutes at room temperature with the brake on.
11. Collect cell pellets in cold EpiCult® Biological Medium (Catalog #07800) and a hematocytometer. Alternatively, count viable cells using Trypan Blue (Catalog #07505).

2.0 CNS TISSUE

2.1 Dissociation of Primary Embryonic Mouse and Rat CNS Tissue

Application: This procedure has been optimized to generate a single cell suspension for expansion of cells as neurospheres or growth in other neural stem cell assays.

1. Isolate embryonic brains from mouse or rat embryos.
2. Transfer the brains to a 25 mm plate containing PBS plus 2% sucrose to cover brain and dissect according to Protocols for Neural Cell Culture (Ed. Sergey Fedoroff and Arleen Richardson, 2001 Ed. 3, pp 173 - 197).
3. Dissect out striata, cortex, ventral mesencephalon or any desired brain region and place in PBS containing 0.25% collagenase.
4. Collect all tissues in a 14 mL tube, allow tissues to settle and pipette off supernatant.
5. Resuspend tissue in Complete NeuroCult® Medium and section as directed below.
   a. Mouse embryonic tissue: 2 mL "Complete" NeuroCult® NEC Preculture Medium (Mouse; Catalog #05702) for dissected tissue from up to 25 embryonic brains.
   b. Rat embryonic tissue: 1 mL "Complete" NeuroCult® ND A Preculture Medium (Rat; Catalog #05711) for dissected tissue from up to 25 embryonic brains.
6. Using a pipette, triturate the tissue until a single cell suspension is obtained.
   a. For mouse embryonic tissue, use a fire-polished glass pipette and tritrate 5-10 times.
   b. For rat embryonic tissue, use a 1 mL pipette with sterile plastic tip and triturate 5-10 times.
7. Add 1 mL "Complete" NeuroCult® Preculture Medium (as in Step 5) to the single cell suspension, mix and leave for ~1 minute to allow the undissociated pieces of tissue to settle.
8. Transfer supernatant to a new sterile 14 mL tube. Discard undissociated tissue.
9. Centrifuge supernatant at 250 x g for 3 minutes with the brake on. Discard supernatant.
10. Resuspend cells in "Complete" NeuroCult® Preculture Medium, as in Step 5.
11. Count viable cells using Trypan Blue (Catalog #07505) and a hematocytometer.

2.2 Dissociation of 4 Day Old Postnatal Mouse CNS Tissue or Adult Mouse or Rat CNS Tissue

Application: This procedure has been optimized to generate a single cell suspension for expansion of cells as neurospheres or growth in neural stem cell assays.

1. Transport human mammary tissue from the operating room on ice in sterile EpiCult® Biological Medium (Catalog #05601) supplemented with 5% fetal bovine serum (FBS; Catalog #06100) and place into a 14 mL or 50 mL centrifuge tube. Approximately ~2.5 mL of the EpiCult® Biological Medium/Collagenase/EDTA/FBS solution will be required for every 2 mammary glands to be dissociated.
2. Rewash mammary glands and transfer to a sterile glass petri dish. Mince with scissors in a cross-wise pattern until glands are rendered to a paste. Transfer the mammary tissue to the tube containing EpiCult® Biological Medium/Collagenase/EDTA/FBS and incubate 6-8 hours at 37°C with occasional pipetting and vortexing.
3. After dissociation, count the cells at 350 g x 5 minutes with the brake on and discard the supernatant.
4. Resuspend the pellet in a 1:4 mixture of ammonium chloride (NH4Cl; Catalog #07801) and cold Hanks’ Balanced Salt Solution Modified (Catalog #03715) supplemented with 2% FBS (Catalog #04000) and centrifuge at 350 g x 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 3.2.

3.0 MMABRODY TISSUE

3.1 Dissociation of Human Mammary Mammary Tissue

Application: These procedures (3.1.1, 3.1.2 and 3.2) 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 NRU (myofibroblastic) repopulating unit assays.
1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in Complete EpiCult® Biological Medium (Catalog #05601) 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 flask (Catalog #27300).
3. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) with 9 parts Complete EpiCult® Biological Medium and add to the minced tissue in the dissociation flask. 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 a sterile aluminum foil.
4. Incubate the dissociated tissue on a rotary shaker at 37°C for ~2-4 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 overflowing liquid from the 50 mL tube and transfer the supernatant to another 50 mL tube. The cell pellet (‘A’ pellet) is highly enriched for epithelial organoids.
7. To generate a single cell suspension from the ‘A’ pellet, please refer to Section 3.2.
8. Centrifuge the supernatant at 200 x g for 3 minutes with the brake on and discard the supernatant. Resuspend cells in medium suitable for subsequent experiments (please refer to the manual for details).
9. Centrifuge the single cell suspension by gently dispensing the cell suspension through a 45 µm cell strainer (Catalog #27300).
10. Count viable cells using Trypan Blue (Catalog #07505) and a hematocytometer.

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

1. Add 1 mL of pre-warmed Trypsin-EDTA (Catalog #07901) to the Collagenase/Hyaluronidase-dissociated mammary cells (pellets from sections 3.1.1, 3.1.2 and 3.2) and resuspend cells. For human tissue, the fraction must be enriched for epithelial cells in the ‘A’ pellet (Section 3.1.1, Step 7).
2. Centrifuge pellets up and down with a P1000 disposable tip for 1-3 minutes.
3. Add 10 mL of cold Hank’s Balanced Salt Solution Modified (Catalog #03715) supplemented with 2% FBS (Catalog #04000) and centrifuge at 350 g x 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 3.2.

3.3 Dissociation of Mouse Mammary Tissue

1. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) with 9 parts Complete EpiCult® Biological Medium (Mouse; Catalog #05601) supplemented with 5% FBS (Catalog #06100) and place into a 14 mL or 50 mL centrifuge tube. Approximately ~2.5 mL of the EpiCult® Biological Medium/Collagenase/EDTA/FBS solution will be required for every 2 mammary glands to be dissociated.
2. Rewash mammary glands and transfer to a sterile glass petri dish. Mince with scissors in a cross-wise pattern until glands are rendered to a paste. Transfer the mammary tissue to the tube containing EpiCult® Biological Medium / Collagenase/Hyaluronidase/FBS and incubate 6-8 hours at 37°C with occasional pipetting and vortexing.
3. After dissociation, count the cells at 350 g x 5 minutes with the brake on and discard the supernatant.
4. Resuspend the pellet in a 1:4 mixture of ammonium chloride (NH4Cl; Catalog #07801) and cold Hanks’ Balanced Salt Solution Modified (Catalog #03715) supplemented with 2% FBS (Catalog #04000) and centrifuge at 350 g x 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 3.2.