TECHNICAL BULLETIN

A guide to 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.

Tissue	Species	Dissociation Method	Reagents Utilized	Page
Bone	Mouse	Mechanical and enzymatic	• Type I Collagenase • Dispase (Catalog #07913)	2
CNS Tissue	Embryonic mouse and rat	Mechanical		2
	Adult or postnatal mouse and rat	Enzymatic	• NeuroCult® Enzymatic Dissociation Kit (Catalog #05715)	2
Mammary	Human, Mouse	Enzymatic	• Collagenase/Hyaluronidase (Catalog #07912) • Trypsin-EDTA (Catalog #07901) • Dispase (Catalog #07913) • DNase I (Catalog #07900)	3
Spleen (for dendritic cells)	Mouse	Enzymatic	• Spleen Dissociation Medium (Catalog #07915)	4
Spleen (for T and B cells)	Mouse	Mechanical		4
Prostate	Human, Mouse	Enzymatic	• Collagenase/Hyaluronidase (Catalog # 07912) • Trypsin-EDTA (Catalog #07901) • Dispase (Catalog #07913) • DNase I (Catalog #07900)	4

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1.0 BONE (MOUSE)

1.1 Isolation of Cells from Mouse Bone and Marrow: Bone Crushing

Application: This procedure has been optimized to isolate cells from mouse bone and marrow prior to mesenchymal cell expansion or CFU-F assays.

1. Isolate and clean femur and tibia aseptically.
2. Crush bones gently in 5 mL PBS with 2% FBS (Catalog #07905) with a sterile mortar and pestle in order to release the marrow (do not grind the bones!).
3. Swirl to mix the bones with the PBS. Allow to sit undisturbed for 1 - 2 minutes in the mortar to allow debris to settle. Use a 5 mL pipette to carefully transfer the suspension (PBS and marrow) through a 70 µm cell strainer into a new 50 mL tube.
4. Add another 5 mL PBS with 2% FBS to the mortar and repeat the gentle crushing of the bones with the pestle. Swirl to mix, let sit undisturbed for 1 - 2 minutes and transfer the cell suspension through the cell strainer into the same tube. Repeat this 2 - 4 times until bone fragments are mostly white.
5. Transfer bone fragments to a 100 mm dish. Add enough 2.5 mg/mL Type I Collagenase (Sigma Catalog #C0130) to just cover the fragments. Let sit for 5 minutes.
6. Chop the bone fragments into fine pieces with a scalpel.
7. Prepare 5.5 mL of Type I Collagenase/Dispase solution where the final concentration of Type I Collagenase (Sigma Catalog #C0130) is 2.5 mg/mL and Dispase (Catalog #07913) is 3 mg/mL. Use PBS to achieve final concentrations. Transfer the bone fragments into the tube containing the Collagenase/Dispase solution.
8. Incubate the bone fragments with gentle agitation at 37°C for 5 minutes in the Collagenase/Dispase solution.
9. Filter the bone fragment solution using a 70 µm strainer and combine it with the solution from steps 3 - 4.
10. Top up the volume in the 50 mL tube to 45 mL using PBS with 2% FBS.
11. Centrifuge at 350 x *g* (~1200 rpm) for 7 minutes at room temperature with the brake on. Resuspend the cell pellet in appropriate medium for desired application.
    a. For cell culture, resuspend in Complete MesenCult® Medium (Mouse; Catalog #05501 and #05502).
    b. For cell separation experiments, resuspend cells in the medium recommended in the cell separation protocol.
12. Place the cells on ice until ready for use.
13. Count nucleated cells with 3% Acetic Acid with Methylene Blue (Catalog #07060) and a hemacytometer. Alternatively, count viable cells using Trypan Blue (Catalog #07050).

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 brains from mouse or rat embryos.
2. Transfer the brains to a 35 mm plate containing PBS plus 2% glucose 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 2% glucose on ice.
4. Collect all tissues in a 14 mL tube, allow tissues to settle and pipette off supernatant.
5. Resupend tissue in "Complete" NeuroCult® Proliferation Medium as directed below.
    a. Mouse embryonic tissue: 2 mL "Complete" NeuroCult® NSC Proliferation Medium (Mouse; Catalog #05700 and #05701) for dissected tissue from up to 25 embryonic brains.
    b. Rat embryonic tissue: 1 mL "Complete" NeuroCult® NS-A Proliferation Medium (Rat; Catalog #05771) for dissected tissue from up to 25 embryonic brains.
6. Using a pipette, triturate the tissue until a fine single cell suspension is obtained.
    a. For mouse embryonic tissue, use a fire-polished glass pipette and triturate approximately 10 times.
    b. For rat embryonic tissue, use a 1 mL pipettor with sterile plastic tip and triturate 5 - 10 times.
7. Add 1 mL "Complete" NeuroCult® Proliferation Medium to the single cell suspension, mix and leave for about 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* (~1000 rpm) for 3 minutes with the brake on. Discard supernatant.
10. Count viable cells using Trypan Blue (Catalog #07050) and a hemacytometer.

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 other neural stem cell assays. This procedure uses the NeuroCult® Enzymatic Dissociation Kit (Catalog #05715). For more information, please refer to the NeuroCult® Enzymatic Dissociation Kit for Adult Mouse and Rat CNS Tissue Technical Manual (Catalog #28930).

1. Add 10 mL of NeuroCult® Tissue Collection Solution to a 100 mm plate.
2. Remove the brains from day 4 pups or perform dissections on the CNS tissue region of interest from adult mouse or rat brains. Transfer the brains or dissected tissue pieces to the prepared 100 mm dish containing NeuroCult® Tissue Collection Solution.
3. Once dissections are complete and the desired number of brains or tissue regions have been collected in the 100 mm dish, remove all the NeuroCult® Tissue Collection Solution and mince the tissue with a scalpel until the tissue homogenate can be pipetted through disposable plastic tips (P200 or P1000).
4. Resuspend the minced tissue in NeuroCult® Dissociation Solution and transfer it into a 14 mL tube.
    a. For day 4 mouse pups, use 3 mL for every 2 brains.
    b. For adult rat brains, use 3 mL for dissected tissue from 6 brains.
    c. For adult mouse brains, use 3 mL for dissected tissue from 8 brains.

5. Incubate the minced tissue in NeuroCult® Dissociation Solution for 7 minutes at 37°C (preferably in a water bath).
6. Add 3 mL of NeuroCult® Inhibition Solution per 3 mL of NeuroCult® Dissociation Solution and mix the tissue suspension gently, avoiding air bubbles.
7. Centrifuge the suspension at 100 x *g* (~700 rpm) for 7 minutes with the brake on.
8. Discard the supernatant and resuspend the pellet in 0.15 mL of NeuroCult® Resuspension Solution.
9. Mechanically dissociate or triturate the digested tissue using a P200 plastic disposable tip until a smooth and "creamy" suspension is achieved.
10. Add 0.1 mL of NeuroCult® Resuspension Solution to bring the volume to approximately 0.3 mL.
11. Pipette the suspension approximately 5 more times with a P1000 disposable plastic tip to achieve a homogenous cell suspension without any remaining chunks of tissue.
12. Add NeuroCult® Resuspension Solution to the tube to a final volume of 10 mL and mix.
13. Wash the cell suspension by centrifugation at 100 x *g* (~700 rpm) for 7 minutes with the brake on.
14. Discard the supernatant and resuspend the pellet with a P200 disposable tip by adding 0.2 mL of NeuroCult® Resuspension Solution.
15. Add NeuroCult® Resuspension Solution to a final volume of 10 mL, mix, and wash the cell suspension by centrifugation at 100 x *g* (~700 rpm) for 7 minutes with the brake on.
16. Repeat steps 14 & 15 once more for a total of 3 washes with the NeuroCult® Resuspension Solution.
17. Discard the supernatant and resuspend the final pellet in 0.5 - 1 mL (depending on the size of the pellet and number of brains or tissues used) of a medium appropriate for subsequent experiments (please refer to the manual for details).
18. Filter the resuspended cell suspension by gently dispensing the cell suspension through a 40 µm cell strainer (Catalog #27305).
19. Count viable cells using Trypan Blue (Catalog #07050) and a hemacytometer.

3.0 MAMMARY TISSUE

Application: These procedures (3.1.1, 3.1.2, and 3.2) are 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.

3.1.1 Dissociation of Human Mammary Tissue

1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in Complete EpiCult®-B Medium (Catalog #05601) supplemented with 5% fetal bovine serum (FBS; Catalog #06100).
2. Transfer the tissue to sterile glass petri dishes, mince with scalpel and then transfer to tissue dissociation flasks (Catalog #27300).
3. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) with 9 parts Complete EpiCult®-B Medium and add to the minced tissue in the dissociation flasks. 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* (~600 rpm) 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 3.2.
8. Centrifuge the supernatant at 200 x *g* (~900 rpm) 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 3.2.
9. The supernatant from the second centrifugation is a single cell suspension enriched for human mammary fibroblasts. To collect these, centrifuge at 350 x *g* (~1200 rpm) 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.

3.1.2 Dissociation of Mouse Mammary Tissue

1. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) mixture with 9 parts Complete EpiCult®-B Medium (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®-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* (~1200 rpm) 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 Hank's Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x *g* (~1200 rpm) 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 3.2.

3.2 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 and resuspend cells. For human tissue, the fraction most enriched for epithelial cells is the "A" pellet (Section 3.1.1, Step 7).
2. Gently pipette up and down with a P1000 for 1 - 3 minutes.
3. Add 10 mL of cold Hank's Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x *g* (~1200 rpm) for 5 minutes

with the brake on. The Hank's + FBS solution is now referred to as HF.
4. Remove as much of the supernatant as possible.
5. Add 2 mL of pre-warmed Dispase (Catalog #07913) and 200 µL of 1 mg/mL DNase I (Catalog #07900). Pipette the sample for one minute with a P1000.
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* (~1200 rpm) 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.

4.0 SPLEEN

4.1 Enzymatic Dissociation

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. In a 60 mm dish, use dissection scissors and forceps to mince 1 - 2 freshly isolated mouse spleens into a homogeneous 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 original tube of Spleen Dissociation Medium.
4. Incubate the tube for 30 minutes at room temperature preferably with constant agitation.
5. Gently pass the tissue several times through a 16-gauge blunt-end needle 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 more minutes.
7. 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.
8. Rinse the empty Spleen Dissociation Medium tube and mesh filter with an additional 10 mL of PBS with 2% FBS.
9. Centrifuge the 50 mL conical screw-cap tube at 350 x *g* (~1200 rpm) for 10 minutes with the brake on and discard the supernatant.
10. Resuspend the cells in PBS with 2% FBS and count viable cells using Trypan Blue (Catalog #07050) or nucleated cells using Acetic Acid with Methylene Blue (Catalog #07060) and a hemacytometer.

4.2 Mechanical Dissociation

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 two 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 slide 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* (~1200 rpm) for 10 minutes with the brake on and discard the supernatant.
8. Resuspend the cells in PBS with 2% FBS and count viable cells using Trypan Blue (Catalog #07050) or nucleated cells with 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 #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* (~1200rpm) 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 #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x *g* (~1200 rpm) for 5 minutes with the brake on.
7. Remove as much of the supernatant as possible.
8. Add 2 mL of pre-warmed Dispase (Catalog #07913) and 200 µL of 1 mg/mL DNase I (Catalog #07900). Pipette the sample for one minute with a P1000.
9. Add 10 mL of cold Hank's 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* (1200 rpm) for 5 minutes with the brake on and discard the supernatant.
10. Count viable cells using Trypan Blue (Catalog #07050) and a hemacytometer