



The Fleischman Lab

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Title	Isolation of Bone Marrow from Major Leg Bones	
Introduction	This protocol explains how to harvest the major leg bones from a mouse for the purpose of isolating hematopoietic cells from the bone marrow. With this protocol, a single mouse typically yields between 25-50 million cells after ACK lysis.	
Materials	<ol style="list-style-type: none">1. R10 medium (RPMI + 10% heat-inactivated FBS + pen/strep)2. 1x ACK buffer3. Isofluorane4. 70% ethanol5. 10 ml syringe6. 27G½ needles7. 50 ml conical tube with a 40 µm cell strainer on top8. 50 ml conical tube containing R10 medium9. Pins10. Dissection board11. Tweezers12. Scissors13. MicroFACS tubes14. Swinging bucket centrifuge15. BD Accuri (for counting cells)16. Optional: R10 + cytokines <p>It is crucial to always maintain sterile technique. Wear a lab coat, work in the mouse hood, and sterilize often!</p>	
Protocol	A. Harvest Bone Marrow	Notes
1.	Euthanize mouse by anesthetizing with isofluorane followed by cervical dislocation.	
2.	Pin mouse to dissection board and spray with 70% EtOH to sterilize.	
3.	Remove fur and skin from legs by lifting skin at the base of each leg with tweezers and cutting away skin across thigh and down to ankle. Peel skin down leg and over foot and firmly tug until it is removed.	
4.	Remove muscle from entire leg so that bone is completely exposed. Blunt dissection with peeling of the muscle works well.	
5.	Isolate the femur and tibia from each leg. Peel off knee cap and snip off top of femur to expose bone marrow.	
6.	Fill 10 ml syringe with R10 and attach a needle.	
7.	Flush bone marrow onto the cell strainer.	

8.	When all bones have been flushed, use plunger of syringe to mush residual cells on top of filter through, pour some R10 on the filter and mush again.	
9.	Discard mouse and all excess tissues according to institutional policy. CLEAN UP.	
10.	Sterilize tools with 70% EtOH and vigorously clean off all tissue and blood.	
B. Process Cells		
1.	Fill tube to 50 ml with R10.	
2.	Centrifuge at 1200rpm (400g) for 5-10 minutes.	
3.	Discard supernatant and resuspend in 3 ml 1x ACK buffer.	
4.	Incubate on ice for 15 minutes.	
5.	Fill tube to 50 ml with R10.	
6.	Centrifuge at 1200rpm (400g) for 5-10 minutes.	
7.	Discard supernatant and resuspend in media of choice (typically R10, but may be R10 + cytokines).	<i>A single non-5-FU-treated mouse typically yields 25-50 x 10⁶ cells. Resuspend in a volume that will approach your desired final concentration.</i>
8.	Transfer 25 µl to a microFACS tube containing 225 µl PBS for counting.	
9.	Count cells on the Accuri by running 20 µl on medium speed. Multiply events/µl by (10,000*total ml) to calculate the total number of cells.	
10.	Bring up to volume that gives the desired concentration.	