**Spin Flush of Murine Bone Marrow Protocol**

*Materials*

1. 0.5 mL tube
2. 1.5mL Eppendorf tube
3. >500uL FACS buffer
4. 18G sterile needles
5. Sterile dissection scissors
6. Sterile dissection tweezers
7. Sterile dissection scalpel
8. Kim wipes

*Preparation*

1. Pierce the bottom of the 0.5mL tube with the 18G needle
	1. Position the needle inside of the 0.5mL tube and push down from the outside
	2. Pry off excess plastic from the piercing with the edge of the needle carefully, or with sterile tweezers
2. Fill the 1.5mL Eppendorf tube with 150-200uL FACS buffer
3. Place the 0.5mL Epitube upright into the Eppendorf tube
	1. The hole will be submerged in the FACS buffer solution
4. Dissect the mouse and remove the femur and tibia
	1. Remove all tissue on the bones using Kim wipes and tweezers
5. Use the edge of the scissors, or scalpel, to pry open the tops of the femur and tibia
	1. The “top” of the bones is the knuckle at which they join at the knee
	2. Use a gentle back and forth motion to pry off the top

*Protocol*

1. Use tweezers to drop the bones into the 0.5mL tube hole-side down
	1. Close the cap of the tube



1. Centrifuge at 10,000xg for 30 seconds at room temperature (tabletop centrifuge)
2. Immediately after centrifugation:
	1. Remove Epitube with bones (bones should be colorless/ translucent) and discard
	2. Resuspend pellet with FACS buffer to avoid clumping
3. [*Optional Filtration*] If working with multiple pellets from the same mouse:
	1. Pool all resuspended cell pellets from the mouse into one 1.5mLtube
	2. Filter pooled cells and FACS buffer through a blue filter top FACS tube (30um) or a 50mL conical tube with a 40um filter on top
		1. Use a P1000 pipette tip

