



HARVESTING OF FEMURS AND TIBIA FROM MICE

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BACKGROUND

- This protocol explains how to harvest the major leg bones from a mouse for the purpose of isolating cell progenitors from the bone marrow. You can see the protocol entitled 'Isolating Bone Marrow-Derived M ϕ 's' for detailed instructions on how to do so.

NOTES

- It is important to maintain sterility throughout this procedure. The entire procedure should be performed in a laminar flow hood and generous amounts of 70% EtOH should be used to sterilize surgical equipment periodically as well as the exterior of the mouse itself.
- This protocol begins after euthanization of the animal. Be sure to follow acceptable procedures at your institution to get to this point.

EQUIPMENT

- 50mL Falcon tube containing 70% EtOH for sterilizing surgical equipment
- 50mL Falcon tube containing R10 medium [recipe below]
- Dissection board
- Pins
- Tweezers
- Scissors

PROTOCOL

1. Pin mouse to dissection board and douse in EtOH to sterilize.
2. 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.
3. Remove muscle from entire leg so that bone is completely exposed. Be very careful not to cut bone as this will compromise the sterility of the bone marrow.
4. The entire leg will be removed. To do so expose hip joint of each leg and cut above the joint, making sure to not remove the top of the femur.
5. Clean bones of any remaining muscle and place in 50mL tube containing R10 media. You can leave foot attached until isolation of bone marrow.
6. Discard mouse and all excess tissues according to institutional policy.

CLEAN UP

- Tools should be sterilized with 70% EtOH and cleaned vigorously of all tissue and blood after use.
- Pins should be discarded into a sharps container

RECIPES FOR MEDIA

R10 medium

RPMI-1640	500 ml
Foetal Calf Serum	55 ml
1 M HEPES	5 ml
Penicillin/Streptomycin	1 vial (5 ml), defrosted at 37°C, containing 10 000 U Penicillin/ml; 10 mg/ml Streptomycin; 200 mM L-Glutamine

Using 5 ml syringes, add the HEPES and Penicillin/Streptomycin to the RPMI through a 0.2µm filter.
Using a 50 ml syringe, add the Foetal Calf Serum through a syringe filter with 0.2µm membrane.
Store at 4°C.