

Bone Marrow Transplantation Protocol

Day 1:

A. Solutions to Prepare

1. Flushing Solution (prep. 50ml for every 1 donor)

- DMEM 45ml
- FBS 5 ml
- Heparin 10U/ml (0.625ml of lab stock)
 - Heparin lab stock is 10ug/uL in DMEM or 0.8U/uL, so 10U/ml is 500U/50ml or 625uL of stock

2. Wash Buffer

- 1X PBS 50ml
- Albumin 1g

3. Suspending solution

- DMEM 50ml
- Albumin 0.5g
- Heparin 5 U/ml (312.5uL of 0.8U/uL stock)

*When needed make stock:

4. ACK Lysis buffer (common bottle in 4°C, aseptically aliquot about 10ml/donor as needed)

- NH₄Cl 4.15g
 - KHCO₃ 0.5g
 - Na₂EDTA 18.6g
 - H₂O q.s. **500ml**
- pH 7.2-7.4

B. Day before experiment (irradiation procedure)

1. Thoroughly clean acrylic, pie-shaped chamber and tape paper towels over the lid to cover the air holes.
2. Place under UV lamp for at least 1 hour to sterilize.
3. Put the chamber into a small black trash bag to transport.
4. Take chamber, pen, paper, ear punch, and get irradiator key from Dorshkind lab.
5. Place mice in chamber slots except for the one exposed slot, place in irradiator on rotating plate and irradiate for desired dose.

6. ***Take transplantation donors back to the lab for use in the morning.

C. Day before the experiment (set up for transplantation)

1. Autoclave tools needed for transplantation.
2. Collect:
 - 10ml syringes (luerlock)
 - 26^{3/8} G needles
 - 70% EtOH
 - sterile culture dishes
 - Gauze
3. Put a sign on the tissue culture hood for the hours you'll be occupying

D. Procedure

1. Prepare all above tools and supplies and get ice.
2. Put all solutions in ice.
3. Pour 15-20 ml of DMEM into sterile Petri dishes.
4. Anesthetize donor mice with Isoflo.
5. Euthanize mice by cervical dislocation.
6. Immerse mice in 70% EtOH for several minutes.
7. Dissect away the skin, muscle and connective tissue from the femurs and tibias.
8. Remove femurs and tibias and place in Petri dishes with DMEM.
9. Take dissected, cleaned bones in DMEM to the tissue culture hood.
10. *Aseptic technique is very important from this step on: cut the very ends off the bones to expose the marrow.
11. Fill a 10 ml syringe with flushing media and flush bone from both ends over a 15 or 50ml tube to catch marrow.
12. Centrifuge at 800-1000 r.p.m. for 5 min. at 4C.
13. Aspirate media, add 10ml ACK buffer and shake. Set on ice for 15 minutes.
14. Wash cells 1X with cold Wash buffer (PBS + Albumin)
15. Resuspend to a single cell suspension in 1 ml Wash buffer. Make a 1:40 dilution, i.e. 5uL cell suspension + 95uL Wash + 100uL Trypan Blue. Put 10 uL of suspension into a hemocytometer. Then, to the cell stock, add 9 more mls to the cells in the 15ml tube to do one more wash while counting the cells during this last spin.
16. Resuspend cell pellet in appropriate amount of Suspending medium to inject desired number of cells/100uL into each recipient.
Ex.: 5×10^6 cells/100uL suspending media/recipient