

BONE MARROW TRANSPLANTATION

(adapted from Lulis lab protocol and Arnaud Monvoisin)

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A- Solutions to prepare

A1- Flushing solution

| | |
|--------------------------|--------|
| DMEM | 225ml |
| FBS | 25ml |
| Heparin | 10U/ml |
| pH 7.3, filter sterilize | |

A2- ACK lysis buffer

| | |
|------------------------------|--------|
| NH ₄ Cl | 4.15g |
| KHCO ₃ | 0.5g |
| Na ₂ EDTA | 18.6mg |
| H ₂ O qsp | 500ml |
| pH 7.2-7.4, filter sterilize | |

A3- Washing buffer

| | |
|------------------|------|
| 1x PBS | 50ml |
| Albumin | 1g |
| Filter sterilize | |

A4- Suspending solution

| | |
|------------------|-------|
| DMEM | 50ml |
| Albumin | 0.5g |
| Heparin | 5U/ml |
| Filter sterilize | |

A4- Heparin solution

| | |
|------------------|-------|
| Heparin | 2000U |
| DMEM | 20ml |
| Filter sterilize | |

B- Procedure

Day 0- Set up:

- 1- Prepare the radiation box:
Clean the mouse pie with soap, dry and spray 70% alcohol
UV sterilize O/N inside the tissue culture hood
- 2- Check mice for irradiation and transplantation
Consolidate the cages

Day 1- Irradiation and set up:

- 1- Get the key to the irradiation room from Dorshkind lab
- 2- Irradiation of the recipient mice- 9.45-9.85 depending on the age, species and the source decay
- 3- Prepare buffers:
 - a- Prepare flushing solution, filter sterilize, keep at 4°C
10ml for each bone (tibia + femur) + extra (~20ml)
 - b- Prepare 1X PBS, filter sterilize, keep at 4°C
 - c- Prepare suspending medium, filter sterilize, keep at 4°C
 - d- Be sure to have needles 26G ^{3/8}(or 27), 70% EtOH, sterile Petri dishes, 15 ml falcon tubes
 - e- Sterilize dissecting tool

Day 2- Bone marrow transplantation:

- 1- Clean the area for mice dissection
- 2- Prepare 70% EtOH
- 3- Anesthetize donor mice with isoflurane vapors
- 4- euthanize the mice by cervical dislocation
- 5- sterilize the mice by immersion in 70% EtOH (20sec)
- 6- Dissect the skin first
- 7- Change the scissors and dissect the muscle around the tibia and the femur
- 8- Twist the bones to separate them
- 9- Cut each part of the bones and flush with the syringe containing 10ml of cold medium
- 10- Centrifuge bone marrow cells at 900rpm/5min/4C (w/ the centrifuge in the tissue culture room) or 1800rpm/5min/4C (w/ the Sorvall centrifuge)
- 11- Lyse red blood cells with 10ml cold ACK, shake and wait 15 min on

ice, centrifuge

12-Wash bone marrow cells (pellet) with 10ml cold wash buffer, spin

13-Resuspend the cells with 1ml of wash buffer, cells go through cell strainer, use 2ml more wash buffer to rinse/transfer leftover cells (1ml each, step by step)

14-Add 7ml wash buffer to filtered cells to make total volume 10ml, spin

15-Add 1ml of wash buffer to the pellet, take 10ul, add to 90ul of wash buffer plus 100ul of Trypan Blue*, mix, take 10ul and count cells

16-While counting, pellet cells again

17-Resuspend cells in an appropriate volume of suspending solution

18-Inject the cells into tail vein (the tail was previously warmed in hot water)

- * The volume of Trypan Blue can be adjusted to avoid excess staining
- Typically, 1 million - 2 million cells are injected for each mouse.
- Volume of each injection - 100µl