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Bone Marrow Transplant (BMT)

Materials needed:

Washing Medium (WM) = IMDM +1% FCS

MACS buffer (MBF) = 500ml PBS + 2ml FCS + 2.5ml 0.5M EDTA pH8

Petri dishes, razor blades/scalpel, 10ml syringes, 25G needles, 50ml conicals, ACK lysis buffer, 70um cell strainers, Miltenyi LS columns and MACS magnet

Antibiotic water which consists of 10 ml Bactrim (sulfamethoxazole and trimethoprim) per liter of water that needs to be given to recipient mice at leat two days before the irradiation and bone marrow transplantation and for 2 weeks after (without this survival is very low)

The irradiation needs to be done for 1000 Rads to achieve complete replacement and this can be done either the night before or a few hours before BM injection

BM isolation

Outside of hood:

1. Wash mouse with 70% EtOH and peel back lower body skin.
2. Cut spine superior to pelvic girdle with scalpel or razor blade. Peel skin off to tail. Cut off legs between spine and hips. Place legs in petri dish.
3. Carefully cut away muscle, tendons and ligaments leaving hip, knee and ankle joint intact. Cut away fibula. Cut foot inferior to ankle joint. Place femur and tibia with joints intact in petri dish filled with WM on ice.

In sterile hood:

1. Cut femur immediately below hip joint and immediately above knee joint with razor blade in empty petri dish.
2. Flush bone marrow with cold WM with a 10ml syringe and 25G needle 1-2 times from each side, or until bone turns white into third petri dish filled with WM.
3. Cut tibia immediately below knee joint and immediately above ankle joint as in step 1.
4. Flush bone marrow as in step 2.
5. After all bones have been flushed, pipette soup up and down several times with 10ml syringe (no needle) until all the cells are homogenized and no marrow sausages (red aggregates) are visible.
6. Pipette entire suspension into 50ml conical(s).
7. Centrifuge at 1320rpm for 10min at 4 °C.
8. Pour off supernatant.

9. Add 5ml cold ACK lysis buffer and pipette up and down for 30sec and fill immediately with cold MBF to 40ml.
10. Centrifuge at 1320rpm for 10min at 4 °C.
11. Pour off supernatant.
12. Resuspend cell pellet with 10ml cold MBF and filter through 70um cell strainer into new 50ml conical on ice.
13. Count cells using hemocytometer: e.g. 1:40 dilution – 5ul cells into 195ul trypan blue → pipette 10ul onto hemocytometer under cover slip: #cells/4 squares x 40(dilution factor) x volume(ml) x 10,000(1ml/10ul) = X$(10)^7$ cells.
14. T cell deplete bone marrow using CD90+ MACS beads and collect the T cell depleted marrow.
15. Centrifuge at 1320rpm for 10min at 4 °C.
16. Pour off supernatant.
17. Resuspend cell pellet in 90ul MBF for every 10^7 cells. Add 10ul CD90+ MACS beads for every 10^7 cells. Incubate for 18min on ice.
18. Fill to 40ml with MBF. Centrifuge at 1320rpm for 10min at 4 °C. While spinning, set up LS column(s) by placing on MACS magnet and rinsing with 3ml MBF. Discard flow-through.
19. Pour off supernatant.
20. Resuspend in 500ul for every 10^8 cells.
21. Load suspension onto column(s).
22. Collect flow-through in 15ml conical on ice.
23. Rinse column(s) 3 times with 3ml MBF collecting flow-through in same 15ml conical.
24. Count cells in flow-through as in step 13.
25. Centrifuge at 1320rpm for 10min at 4 °C.
26. Pour off supernatant.
27. Resuspend cells in sterile PBS at at least 10x10^6 cells per 200ul in order to inject 10x10^6 cells per mouse.

BM Injection

BM cells are kept on ice and injected to achieve at least 10x10^6 T cell depleted cells injected per mouse, 200ul is a nice volume but can give up to 1 ml per mouse without problems

Continue antibiotic water for 2 weeks changing with fresh water 2X per week