General Surgery Labs SOP
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Kent Zettel

DC cell isolation (Adoptive Transfer Pt 2)

Stain Buffer/MACs Buffer

- Completely dissolve 0.7444g EDTA in 900ml PBS
- Add 5g Bovine Serum Albumin to mixture
- Bring to 1L using PBS
- pH to 7.2
- Filter and store at 4˚C for 2 weeks

This is a summarization of the Milenyi MACS magnetic bead CD11c isolation protocol:

1. Smack flask 3 times to remove the loosely adherent DCs – have the media swish across the bottom of the flask hard with the smacks
2. Rinse flask bottom with the media inside the flask
3. Place media in 50ml conical
4. Centrifuge at 2000 rpm for 5 mins and decant supernatant
5. Compine cells from all the conical into one, using PBS or stain buffer to rinse and resuspend
6. Centrifuge at 2000 rpm for 5 min and decant supernatant
7. Add 400ul stain buffer to conical
8. Add 50ul FC block
9. Add 100 ul magnetic beads for 2-3 flasks (150ul for 4 flasks)
10. Incubate 30 mins at 4-8˚C on a shaker
11. Add stain buffer to 30ml
12. Centrifuge at 2000 rpm for 5 mins. (While waiting for centrifuge, perform steps 15 and 16)
13. Decant supernatant. Aspirate all remaining supernatant using a 200ul pipet tip prior to inverting the conical vial.
14. Add 500ul stain buffer.
15. Add LS column (with a yellow filter if injecting into animals) to the magnet on the stand. Place a 15ml conical underneath it without touching the tip of the column
16. Add 3ml stain buffer to the magnet. Allow to filter through column until no longer dripping. (Can be done during step 12)
17. Add cells to column. Allow to filter through until no longer dripping
18. Add 3 ml stain buffer. Allow to filter until no longer dripping. Repeat 3times (a total of 12.5mls of stain buffer will have run through the column: 3+0.5with cells+3+3+3 = 12.5)
19. Remove the column from the magnet – place on top of a fresh 15ml conical
20. Add 5ml stain buffer. Allow to filter until no longer dripping
21. Add 5ml stain buffer. Push through column with plunger supplied in column package
22. Count cells.

For injection into mice add $10x10^6$cells in 300ul LRS for each mouse 1 day prior to trauma.