

Isolation of lymphocytes from pancreas, LN, spleen and thymus

Method

1. Perform cardiac perfusion (40 ml PBS/mouse) and harvest pancreata, PLN, CLN, thymi & spleen. **Make sure to pick pancreas clean of all LN.**

NB. Process all tissues in the same manner (cutting with scissors into 1 mm pieces and placing in digestion buffer etc).

2. Prepare **fresh** digestion buffer:
 - DMEM
 - collagenase Type IV [Gibco # 17104-019] @ 1 mg/ml
 - DNaseI [Sigma # DN25] @ 10 U/ml
 - 1% BSA [Sigma # A7409].
3. Prepare single cell suspensions by cutting up tissues using scissors (1 mm pieces of pancreas) and placing into freshly prepared digestion buffer as follows:
 - Pancreas – 25 ml in 50 ml tube
 - Thymus - 1 ml in 1.5 ml eppendorf tube
 - LN – 0.5 ml in 1.5 ml eppendorf tube
4. Digest for 20 min at 37⁰C at 150 rpm submerged in a shaking waterbath.

For pancreata:

5. Filter digest (blue cell strainer with 40 µm nylon; BD ref 352340) into fresh 50 ml tube. Force undigested pieces through cell strainer with the plunger of a 3 ml syringe. Add DMEM (through strainer) up to 40 ml.
6. Centrifuge at 1500 rpm for 10 min.
7. Resuspend pancreas pellet in 2 ml DMEM and pass through nitrocellulose membrane into 5 ml round bottomed polystyrene tube.

For other tissues:

8. Resuspend undigested pieces of tissue using pipette (& shortened 1 ml pipette tip) in the eppendorf tube until single cell suspension achieved.
9. Filter suspension through nitrocellulose membrane into 5 ml round bottomed polystyrene tube and add 1 ml DMEM/tube before centrifuge at 1500 rpm for 5 min.
10. Resuspend spleen samples in 1 ml ACK lysis buffer for 2 min at RT.

For all tissues:

11. Add 1-2 ml DMEM/tube and centrifuge at 1400 rpm for 5 min.
12. Resuspend in appropriate Ab or control substance.
13. Incubate for 20 min on ice.

If 2⁰ stain required perform steps 14 & 15. Otherwise proceed to step #16.

14. Add 150 µl DMEM per well and centrifuge at 1500 rpm for 5 min.
15. Resuspend samples in apt. 2⁰ Ab and incubate on ice for 10 min.
16. Add 150 µl DMEM per well and centrifuge at 1500 rpm for 5 min.
17. Resuspend in 200 µl DMEM/well.

Alternatively, resuspend cells in 50:50 mix of DMEM:1% formalin/PBS if not running cells right away.

18. Run samples on LSRII.