

Production of Antigen Specific Blasts for T Cell fusion

Day 0: Injection of mice

1. Prepare protein in Complete Freund's Adjuvant.
2. Inject 50 ug protein in 50 ul into the base of mouse tail (sub-cutaneous).

Day 7: Lymph node cells into culture

1. Bleed mice from the tail vein. Store blood at room temperature ~ 10 minutes. Place tube onto ice for > 30 minutes. Spin blood 2000 rpm, 5 minutes (OR spin in the microfuge, ~5000 rpm, 5 minutes). Save serum for use in Click's medium.
2. Remove draining lymph nodes (inguinal & para-aortic). Dissociate cells in sterile Balanced Salt Solution. Spin cells 1X, 1450 rpm, 5 minutes.
3. Wash cells 1X in BSS. Count cells.
4. Prepare Click's with 0.5-1.0% fresh normal mouse serum and antigen (100 ug/ml). Filter complete Click's medium through 0.22 u filter before adding the cells.
5. Resuspend the cells in complete Click's medium to a concentration of 4×10^6 /ml.
6. Plate cells in a 24 well Costar plate, 1.5 ml/well. Incubate in 2-5% CO₂ for 4 days.

Day 11: Purifying blasts for culture in IL-2

1. This procedure is done at room temperature including the ficoll spin. After isolating the blasts the spins can be done at 4°C.
2. Using a Pasteur pipette resuspend cells in Costar wells and transfer to a 50 ml conical tube. Rinse each well with BSS and combine with cells.
3. Spin 1X, 1450 rpm, 5 minutes.
4. Suction off supernatant and resuspend cell pellet in 10 ml BSS + 15% fetal bovine serum.
5. Underlay cells with 3 mls LSM solution (LSM Lymphocyte Separation Medium, ICN Biomedicals, www.icnbiomed.com). Maximum number of cells per gradient should be 10^8 .
6. Spin 1500 rpm, 15 minutes, room temperature.
7. Carefully aspirate off ~ 8 mls of BSS above the interface. Then, using a Pasteur pipette, recover the cells at the interface. Transfer cells to a clean tube containing 10 mls BSS.
8. Spin cells 1X, 1450 rpm, 5 minutes.
9. Wash cells 2X in BSS.
10. Resuspend cells in Complete Tumor Medium. Count cells.
11. Culture cells at 1×10^5 /ml in CTM + IL-2 in a flask.
12. Incubate flask at 10% CO₂, 37°C for 3 days.
13. Three days later: spin cells and use for fusion.