Protocol: Isolation of Mouse Liver Lymphocytes
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10/00

Reagents Needed (all sterile)
1. Sterile PBS (GIBCO) 1X
2. Percoll (Sigma) 100%
3. Heparin (Upjohn) 1,000U/ml
4. RPMI 1640 + 2ME (500 µl of 1000X GIBCO) + 10% heat-inactivated FBS + 100 µl
   50 mg/ml Gentamycin (Gibco).
5. 1X ACK
6. Sterile forceps, dissection tools, tissue culture dishes, mesh screen (BELLCO)
7. ETOH
8. 5CC Syringes
9. Falcon cell strainer (50 CC tube size)

Method
1. Sterilly dissect the entire liver minus the gall bladder - place into a 60 mm petri
   dish.
2. Add 3ml PBS for each liver to be homoginized.
3. Press/homoginize the liver tissue against the mesh screen (bent to make a ramp in
   the dish) with the plunger from a 5CC syringe.
4. Add 7ml PBS per liver to the dish and filter the single-cell suspension into a 50ml
   conical tube (per liver) through another sterile mesh that is shaped into a funnel or a
   Falcon cell strainer (50 CC tube size).
5. Rinse the dish with 10ml PBS and filter into the 50ml tube.
6. Add 10ml Percoll to each 50 ml tube (final =33%).
7. Add 300 µl Heparin to each 50ml tube.
8. Spin 20 min. at 2,000 rpm at room Temp.
9. Aspirate the parenchymal cells and the supernatant. Pellet = RBC and Lymph
10. Combine pellets and wash in 50ml PBS. Spin 1,500 rpm for 5 min. 4°C.
11. Resuspend pellet in 1X ACK (5ml per liver) Incubate RT 10 min.
12. Fill tube with complete RPMI medium and spin 1,500 rpm for 5 min., 4°C.
13. Aspirate and repeat 11 and 12 if pellet still red.
14. Resupsend in 1-10 ml RPMI medium - take sample to Coulter count.
15. Spin 1,500 rpm for 5min. Resuspend cells to desired concentration.