Micro-Techniques of Cell Harvesting in Mice

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Mice are wonderful models of the human immune system. They are used extensively in immunology research and lend themselves easily to undergraduate laboratory curricula and undergraduate research. Micro-techniques of cell harvesting have several advantages when performing studies with mice, including: 1) fewer mice are needed, 2) a single mouse can be monitored throughout the duration of the experiment, and 3) mice need not be sacrificed. The one drawback of these techniques is that studies are limited to peripheral blood analyses. Preliminary results from peripheral blood studies may help to determine the need for further studies for which animals must be sacrificed. We have employed these micro-techniques in our immunology undergraduate research projects and have obtained sufficient quantities of cells to do lymphocyte fluorescent antibody staining, in vitro tissue cell culturing and mitogen stimulated lymphocyte proliferation assays.

1. Before obtaining blood, anesthetize the mouse. Place six cotton balls into a wide mouth jar with a flat top. Under a fume hood, pipette 1.3 ml of Metofane onto the cotton and cover the jar with a watch glass. Place the mouse in the jar and remove the mouse when it has remained inactive for about 2 minutes.
2. Blood is obtained using the orbital sinus technique. Insert a heparinized capillary tube through the medial canthus of the conjunctiva and rotate the tube to sever the orbital sinus plexus. At least two or three tubes of blood should be collected from each mouse.
3. Blood-filled tubes are placed into EDTA-coated eppendorf tubes and blood is allowed to drain from the capillary tubes. Close the eppendorf tube and tap it gently to mix the blood with the EDTA to prevent clotting.
4. Cleanse the eye and fur of the mouse with sterile saline and return the mouse to its cage when awake.
5. Fill fresh heparinized capillary tubes with the collected blood and seal with sealing clay at one end. Spin the blood samples in a hematocrit centrifuge for 5 minutes. This will produce a serum layer, a white cell layer, and a red cell layer.
6. Etch the capillary tube with a file just above the white cell layer and gently break the top of the tube off at the etch mark. Insert the needle of a “tuberculin” syringe into the broken end of the tube and draw the white cells into the syringe.
7. Draw up 500*ul* of sterile phosphate-buffered saline containing 1% bovine serum albumin (PBS-1%BSA). This moves the cells through the needle and keeps the cells from clumping. Remove the needle and dispense the mixture into a 12 ∞ 75 mm capped tube and add 10*ul* of red blood cell lytic agent. This will lyse any red blood cells that were drawn up into the syringe.

8. Gently invert the tube to mix and allow 2 minutes for the lytic agent to have full effect. Centrifuge at 1750 rpm for 5 minutes in a refrigerated tabletop centrifuge. Pour off the supernatant taking care not to disturb the thin film of cells on the bottom of the tube.
9. Add 1 ml of PBS-1%BSA to the tube and suspend the cells by shaking the tube gently. Centrifuge at 1750 rpm for 5 minutes in a refrigerated tabletop centrifuge.
10. Pour off the supernatant carefully and dab the final fluid droplets with a Kimwipe while the tube is inverted. Resuspend the cell pellet in an appropriate volume of isotonic solution for further analysis.