

Preparation of Immediately Centrifuged Rat Serum

Rats will be used to prepare rat serum, which will be used to culture mouse embryos. For preparation of rat serum, a published protocol will be followed (Sturm, K. and Tam, P.P.L. (1993). Isolation and culture of whole postimplantation embryos and germ layer derivatives. *Methods Enzymol.* 225, 164-190). Rats are anesthetized by placing a rat on a platform over ether in a glass desiccator. Diethyl ether is preferred over other anesthetics because the dosage can be regulated easily and residual ether is removed from the serum during heat inactivation of the serum. The anesthesia is performed in a chemical fume hood clear of equipment and other potential fire hazards. Anesthesia is monitored by foot and tail pinch and blink reflex. The etherized animal is placed on its back and maintained under anesthesia by bringing a tube containing a piece of ether-soaked tissue over its snout. The abdomen is sprayed with 70% ethanol and cut open. The viscera are displaced to one side, and the tissues covering the dorsal aorta are reflected with forceps. The abdominal aorta is punctured with a 19 gauge butterfly needle and the blood is collected through tubing directly into Vacutainer tubes. 12-15 ml of blood is collected from each rat, using two tubes. Blood collection takes about 2 minutes and exsanguination is complete.

Rats are "surplus male rats" from Zivic-Miller, RSD750, Sprague-Dawley outbred, about \$8.00 each. We don't house the mice, just take them from the crate the day or day after they arrive. The vacutainers and butterflies are from Becton Dickinson: SST blood tube, red/gray, gel/silica, pack of 100 # 367985, \$50, and Blood collection set, 19 gauge needle, box of 50, # 364919, \$55.13.

The blood tubes are immediately centrifuged for 5 minutes at room temperature at 1,800g in a table top centrifuge. If the serum is amber in color without red tint, it is pooled, re-centrifuged, and heat denatured for 30 minutes at 56°C.

6.5 day embryos are cultured in 50% rat serum 50% MEM. 7.5 day embryos in 75% rat serum 25% high glucose DMEM.