Blood collection and plasma and serum preparation from mouse retro-orbital plexuses.v1

Procedure

1. Prepare all of sterile surgical equipment, including the scissors, small forceps, and 1.5-ml polypropylene microcentrifuge tubes coated with EDTA (see below).
2. Mark and weigh the mouse and recorded it.
3. Put the mouse into an induction chamber to anesthetize the mouse through inhalation anesthesia with isoflurane.
4. Grasp its skin of the neck and the tail with one hand.
5. Cut the mouse's whiskers and the hair around both eyes with scissors.
6. Press the edge of one eyeball with scissors to push it out, cut the whole eyeball and pull it out totally. Blood should immediately flows from the retro-orbital plexuses.
7. Collect the blood into 1.5-ml EDTA coated Eppendorf tubes containing 8 ul of 0.5 M EDTA (See below for the coating procedure) that is chilled on ice and gently mix.
8. Gently mix the blood and anticoagulant and store on ice.
9. Estimate the volume of the blood and add additional amount of 0.5 M EDTA so the final concentration of EDTA is 5 mM. e.g. to 1 ml of blood, you need to add additional 2 ul of EDTA.
10. Centrifuge the sample for 15 min at 3,000 rpm (1500 × g) at 4°C. DO NOT use brake to stop centrifuge.
11. Carefully transfer the supernatant (plasma) to a 0.5 ml-Eppendorf tube packed with glasswool and a hole at the bottom and spin at 3,000 rpm for 15 sec at 4°C.
12. Trasfer the sample to a filter unit of 0.22 micron and spin at 5,000 rpm for 1 min at 4°C to collect the filtered plasma.

13. For serum collection, collect blood into regular 1.5-ml Eppendorf tubes and store at room temperature for 1 hr, and perform Step 10 to obtain serum.
14. The plasma (or serum) is ready for analysis. If necessary, store at −20°C or −80°C.

Collection tube preparation

Add 1.5 ml of 0.5 M EDTA to an Eppendorf tube and transfer to other tubes in sequence on the day of sample collection.