Isolation of crude nuclei from rat liver

1. Wash 50 gram of liver once with cold PBS.

2. Cut the liver into small pieces (c.a. 3 mm cubic) with scissors on ice.

3. Add 100 ml of ice-cold homogenize buffer (20 mM potassium phosphate, pH 7.2, 0.25 M sucrose, 4 mM $CaCl_2$ 4 mM beta-mercaptoethanol).

4. Homogenize in a Dounce homogenizer in ice (usually 15-20 up and down strokes).

5. Layer 20 ml of the homogenate on 10 ml of sucrose cushion (0.88 M sucrose, 3 mM $MgCl_2$, 4 mM $CaCl_2$).

6. c.f.g. at 3,000 rpm (1,610 x g) for 10 min at 4 °C.

7. Take a pellet of crude nuclei.

8. Resuspend the pellet in 10 vols. of homogenize buffer (20 mM potassium phosphate, pH 7.2, 0.25 M sucrose, 4 mM $CaCl_2$ 4 mM beta-mercaptoethanol).

9. Layer the suspension over an equal volume of 0.88 M sucrose solution.

10. c.f.g. at 3,000 rpm (1,610 x g) for 10 min at 4 °C to obtain a pellet of clear nuclei.