
Today’s Protocol

NOTE: All solutions and glassware should be precooled to 0 to 4°C and kept on ice throughout the day’s work. Following your professor’s instructions, students should prepare an ice-cold stage on which liver tissue can be properly handled.

1. **Tissue Preparation.**
   a. Add 15 ml ice-cold Nuclear Isolation Medium (NIM) to the pre-cooled glass homogenization mortar and keep on ice.
   b. Each pair of students will be given one-half of a small rat liver. On the ice-cold stage, finely mince the liver with a razor blade to no more than ~ 25 mm³ (rough cubes not greater than 3 mm on each side = 25 mm³).
   c. Pool your minced liver with the student group across from you and, using a plastic spoon, transfer the tissue to the glass homogenization mortar. Make sure all liver pieces are submerged in the NIM, and keep on ice.

2. **Homogenization.** Under instructor supervision, homogenize the minced tissue on ice with a Potter-Elvehjem homogeniser, maximum speed, 6 - 7 strokes of the pestle.

3. **Filter the Homogenate.** Pour the homogenate into a small beaker on ice through 4 layers of cheesecloth to filter out clumps of connective tissue and unhomogenized chunks of liver tissue. You only need to collect about 10 ml of filtered homogenate.

4. **Prepare Your 25% Iodixanol - Homogenate Sample.**
   a. Transfer 4 ml of your filtered homogenate to a pre-cooled 15 ml conical centrifuge tube.
   b. Add 4 ml **50% iodixanol** to the 15 ml centrifuge tube and invert 3X to mix with the homogenate. This is your **25% iodixanol-homogenate sample**. Keep on ice until needed.
5. **Prepare Your Three-layer Density Gradient.** Prepare a three-layer 4 – 3 – 2 iodixanol density gradient in the following way:
   a. To an ice-cold 10 ml centrifuge tube, carefully transfer 2 ml of 35% iodixanol to the very bottom of the centrifuge tube.
   b. Next, carefully layer 3 ml of 30% iodixanol on top of the 35% iodixanol.
   c. Next, carefully layer 4 ml of the 25% iodixanol-homogenate sample on top of the 30% iodixanol.

6. **Centrifuge to Isolate the Nuclei.**
   a. Centrifuge the tubes in a swinging-bucket rotor at 9,000 rpm (10,000 x g) for 25 minutes at 4°C.
   b. When the centrifugation is complete, study the tubes and locate the band of nuclei resting on top of the 35% iodixanol layer.
   c. Using a fresh Pasteur pipet, decant and dispose of the top 25% iodixanol-homogenate layer.
   d. Using a second fresh pipette, decant and dispose of the second 30% iodixanol layer. Be careful NOT to remove any of the nuclear band.
   e. With a third fresh pasteur pipette, carefully remove the nuclear band and transfer it to a single 2 ml microcentrifuge tube. Expect to harvest approx. 1 ml nuclei from your centrifuge tube.

7. **Add the fluorescent DNA stain and incubate.**
   a. Add 100 μl of the Hoechst fluorescent DNA stain to the microcentrifuge tube containing the isolated nuclei.
   b. Incubate the tube in a shaking shaking platform at RT for 15 minutes.

8. **Pellet the Nuclei in a Microcentrifuge.**
   a. Centrifuge the tubes in a microcentrifuge at 3,000 RPM for 10 minutes.
   b. Following the spin, look for a small white nuclear pellet. Decant the supernatant without disturbing the pellet, add 1 ml fresh NIM, gently draw the nuclei back and forth into the Pasteur pipet to mix.

9. **Prepare slides, observe using fluorescence microscopy.**