Succinate Dehydrogenase from Chicken Livers

Extraction of Chicken Liver Mitochondria

1. Label 13 microfuge tubes and place them on ice:
    a. 6 tubes – “1.7P” with your initials
    b. 6 tubes – “14 P” with your initials
    c. 1 tube – “Mito Prep” with you initials

2. Weight the exact mass of chicken livers. You need approximately 2 g.

3. Use a razor blade to finely mince the chicken liver in the weigh boat while it is sitting on ice.

4. Use the razor blade to transfer the minced chicken liver into an ice-cold mortar, and add 5 mL of ice cold extraction buffer ([10 mM HEPES (pH 7.5); 0.25 M sucrose]. Use the pestle to crush the chicken liver into homogeneous mixture containing no lumps. You should grind for approximately 4 minutes. Add 5 mL of ice cold extraction buffer ([10 mM HEPES (pH 7.5); 0.25 M sucrose]. Mix gently.

5. Pour the chicken liver homogenate through a cheese cloth lined funnel into a 50 mL conical tube sitting in ice. Pour an additional 5 mL of extraction buffer ([10 mM HEPES (pH 7.5); 0.25 M sucrose] through the cheese cloth.

6. Fill as many of the microfuge tubes labeled “1.7P” as you can with filtered homogenate. Be sure to leave approximately ¼ of the volume as headspace.

7. Centrifuge the tubes in balanced positions for 5 minutes at 1,700 rpm. Remove the tubed to ice.

8. Carefully, transfer the supernatant into microfuge tubes labeled “14P”. Be sure not to take any pelleted material and to leave approximately ¼ of the volume as headspace in the new tubes. The pellet should contain cell membrane and nuclear material.

9. Centrifuge the tubes in balanced positions for 10 minutes at 14,000 rpm. Remove the tubed to ice. The mitochondria should be in the pellet.

10. Resuspend the pellets in a total of 1 mL of ice-cold extraction buffer ([10 mM HEPES (pH 7.5); 0.25 M sucrose]. You will need to resuspend the pellet from the first tube, transfer the solution to the next tube, and continue. Keep the solution on ice.

11. Add Thesit detergent to a final concentration of 30 g/L. Incubate on ice for 30 minutes.

12. Spin balanced tubes for 10 minutes at 14,000 rpm. Collect the supernatant in a clean tube.