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Mitochondrial respiration measurement

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Method Article

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Abstract

Introduction

Mitochondrial respiration measurement modified for _Drosophila_.

Procedure

1) Isolate mitochondria by gently crushing 40 to 80 flies in a 10 ml Kontes homogenizer with 7 strokes of the pestle in 3 ml homogenization buffer consisting of 225 mM mannitol, 75 mM sucrose, 10 mM MOPS, 1 mM EGTA, 0.5% BSA, pH 7.2 at 4°C 38. 2) Filter the extracts through 8 layers of cheese cloth, and then centrifuge at 300 x G for 3 min in a Beckman Avantis-J25. 3) Centrifuge the supernatant at 6000 x G for 10 min to obtain a mitochondrial pellet. 4) Measure the mitochondrial protein concentration by the Bradford method, using Bio-Rad reagents, corrected for the BSA content in the homogenization buffer. 5) Determine the respiration rates by measuring oxygen consumption using a Clark-type electrode and metabolic chamber containing 0.65 ml of reaction buffer consisting of 225 mM mannitol, 75 mM sucrose, 10 mM KCl, 10 mM Tris-HCl, 5 mM KH_2PO_4, pH 7.2 at 25°C. 6) Calculate the mitochondrial ATP production rates from ADP consumption rates during state III respiration.