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Cytochrome c oxidase assay

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- 1 Third instar larvae were ground for 10 s in 100 μ L of ice-cold homogenization buffer (50 mM phosphate buffer containing 0.05% Tween-80) using a Kontes pellet pestle motor.
- 2 Homogenates were then diluted with an additional 500 μ L of homogenization buffer.
- 3 To remove cell debris, samples were centrifuged at 2000 *g* for 5 min at 4 °C.
- 4 The supernatant was diluted 1 : 10 and 40 μ L aliquoted into six sample wells of a 96-well plate.
- 5 At the time of assay, 160 μ L of reduced cytochrome *c* (50 μ M) was added to each sample well.
- 6 The plate was assayed in a Molecular Devices SpectraMax 384 Plus microplate reader, reading every 10 s at 550 nm for 5 min.
- 7 Oxidation of cytochrome *c* was indicated by a decline in the OD at 550 nm (extinction coefficient = 29.5).

- 8 Maximum slope of the optical density plot is calculated by the plate reader and is taken as $V_{\max}$ for the reaction.
- 9 A no-cytochrome *c* control was included and the protein concentration of each sample was determined using the Bio-Rad D_C Protein Assay method.