# Cytochrome c oxidase assay

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Aw WC, Towarnicki SG, Melvin RG, Youngson NA, Garvin MR, Hu Y, Nielsen S, Thomas T, Pickford R, Bustamante S, Vila-Sanjurjo A, Smyth GK, Ballard JWO (2018) Genotype to phenotype: Diet-by-mitochondrial DNA haplotype interactions drive metabolic flexibility and organismal fitness. PLoS Genet 14(11): e1007735. doi: 10.1371/journal.pgen.1007735

PROTOCOL STATUS

**Working**

1 Third instar larvae were ground for 10Â s in 100Â ÂµL of iceâ€cold homogenization buffer (50Â mmÂ phosphate buffer containing 0.05% Tweenâ€80) using a Kontes pellet pestle motor.Â

2 Homogenates were then diluted with an additional 500Â ÂµL of homogenization buffer.

3 To remove cell debris, samples were centrifuged at 2000Â *g*Â for 5 min at 4Â Â°C.Â

4 The supernatant was diluted 1Â :Â 10 and 40Â ÂµL aliquoted into six sample wells of a 96-well plate.

5 At the time of assay, 160Â ÂµL of reduced cytochrome*Â c*Â (50Â Âµm) was added to each sample well.

6 The plate was assayed in a Molecular Devices SpectraMax 384 Plus microplate reader, reading every 10Â s at 550Â nm for 5Â min.

7 Oxidation of cytochrome*Â c*Â was indicated by a decline in the OD at 550Â nm (extinction coefficientÂ =Â 29.5).

8 Maximum slope of the optical density plot is calculated by the plate reader and is taken as $V_{max}$Â for the reaction.

9 A noâ€cytochrome*Â c*Â control was included and the protein concentration of each sample was determined using the Bioâ€Rad $D_C$Â Protein Assay method.

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