Oxidative stress

PLOS Genetics

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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 10 Larvae were disseted on ice and placed in 100uL of ice-cold mitochondrial isolation buffer

2 The larvae were homogenized by 80 strokes up and down using sterile kontes pellet pestles

3 The homogenate was pipette into the syringe (with 2cm2 of gauze pad), followed by 400uL of isolation buffer

4 Gently press through the gauze into a clean 1.5mL tube

5 Filtered homogenate was centrifuge at 1500g for 8 min.

6 The resulting brown grey pellet was washed with 200uL of isolation buffer 1 to 2 times to remove the lipid.

7 The final mitochondrial pellet was re-suspend in 20uL of mitochondrial isolation buffer

8 5uL of mitochondria solution was added into 180uL respiration buffer in 96 well plate and stirer by magnatic stirir

9 Add 5uL of 2.20mM ampliflu red (55uM final conc) + Â 10uL of Â 0.02U/uL(20U/mL) horseradish peroxide (1U mL-1 final conc)

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10 After the reaction was initiated, substrate were added as follows (allowing a period of stabilization between each step (Pyruvate + L-poline + Malate each 10mM, ADP 5mM, Rotenone 0.5uM and Antimycin A 2.5uM)

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