Citrate synthase activity

PLOS Genetics

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Cage Studies

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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 For in vitro assays intact mitochondria were isolated, and citrate synthase activity measured from female third instar wandering larvae

2 Set the plate reader at 412nm on a kinetic program : Duration Â 1.5 minute ; Interval 10 seconds

3 Transfer 93 uL of isolated mitochondrial to a 96 well plate to make a final concentration of 2.0ug/mL

4 Add in 1uL of Acetyl CoA (30mM) and 1uL of DTNB (10mM)Â

5 Follow the absorbance of the reaction mixture for 1.5 minutes to measure the baseline reaction, endogenous levels of thiol or deacetylase activity

6 Add 5uL of 10mM oxaloacetate to each well to initiate the reaction. In order to start the reaction in all well simultaneously as possible, use multichannel pipette.

7 Shake the plate for 10 second before reading absorbance. Activity was measure at 412nm (molar extinction coefficients for DTNM were 13.6 L mol-1 cm-1)