Enzymatic activity of electron transport system Complexes I

PLOS Genetics

Wen Aw[1]

[1]z3314717@unsw.edu.au

dx.doi.org/10.17504/protocols.io.rq5d5y6

Cage Studies

Wen Aw

EXTERNAL LINK

https://doi.org/10.1371/journal.pgen.1007735

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

nprot.2012.pdf

PROTOCOL STATUS

Working

1 Dilute the mitochondrial into 0.4ug/uL with distilled water

2 Add 5uL of diluted mitochondria to 73uL of distilled water and incubate for 1 minute

3 Prepare buffer mix= 100uL of potassium phosphate buffer (0.5M, pH 7.5), 60uL of fatty acid free BSA (50mg/mL), 30uL of KCN (10mM), and 10uL of NADH (10mM)

4 Add 20uL of buffer mix

5 Prepare in parallel, a separate well containing the same quantity of reagent and sample with the addition of 10uL of 1mM rotenone

6 Start the reaction by adding 1uL of 10mM Ubiquinone. Measure the reading for 3 min