G6PD Activity

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 Homogenize 2 larvae in 40uL of lysis buffer (20mM Tris-HCL, pH8.0, 3mM magenesium chloride, 1mM EDTA, 0.02% B-mercaptoethanol, Protease inhibitor and 0.1% Triton X100)

2 Centrifuge at 16,000g for 15min at 4 degree celcius and collected the supernatant

3 Protein concentration is determined by Bradford Assay

Standard Preparation

4 Prepare NADPH standard ranges from 0-0.3mM in buffer

5 Buffer containing 5mM of both G6P+ 6PGL, 2mM NADP+

6 Buffer containing Â 5mM of 6PGL, 2mM NADP+

Assay Running

7 Added NADP+ into all well (2.5uL of 80mM +37.5uL of Tris Buffer)

8 Added 10uL of sample to make up a final volume of 50uL

9 Initiate reaction by adding 50uL of substrate (G6P+6PGL (both 5mM final concentration) or 6PGL (5mM final concentration)

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