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Haemolymph extraction of adult Drosophila V.3

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This is a very simple protocol showing how to extract haemolymph from adult Drosophila melanogaster. (Based on protocols from Sigma Aldrich).

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protocol

Damrau C, Toshima N, Tanimura T, Brembs B, Colomb J, Octopamine and Tyramine Contribute Separately to the Counter-Regulatory Response to Sugar Deficit in . Frontiers in Systems Neuroscience doi: 10.3389/fnsys.2017.00100

minor corrections and clarifications to the protocol's wording and description

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Fly preparation

1 Punch three holes in a 0.5ml Eppendorf cap and put it into a 1.5ml Eppendorf cap with removed lid.

2 Remove the flies' wings, spear the fly's thorax with a needly.

3 Collect 20 speared flies in the 0.5ml Eppendorf cap with holes, on ice.

4 Centrifuge the 0.5ml Eppendorf cap within the 1.5ml one (1 min, 5000 rpm, at 4°C).
00:01:00

5 Discard the 0.5ml Eppendorf cap, collect the extracted hemolymph with a fine capillary.

6 Record the amount of haemolymph (to fill up 0.5µl you need around 50 flies).

Enzymatic procedure

7 Add 19.5µl cold PBS to 0.5µl haemolymph.

8 Add 10µl of this mixture to 30µl Citrate Acid Buffer and 10µl of a 3% Trehalase-Citrate acid buffer solution.

9 Incubate over night at 37°C.
18:00:00

10 Add 50µl Tris Buffer.

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11 80µl of this mixture are added to 156.8µ Glucose oxidase (aliquot in the freezer) and 3.2µl o-Dianisidine (freshly added from the fridge).

12 Incubate for exactly 30 min at 37°C.
00:30:00

13 Stop reaction by adding 160µl Sulfuric Acid.

14 Measure at 540nm at the (nanoDrop) spectrometer.

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