Sugar content in haemolymph

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Abstract

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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Guidelines

Materials:
-0.5 µl capillaries with adequate suction cup
-0.5ml and 1.5ml Eppendorf cap
-Ice
-Peaked stylus
-2 forceps (or scissors) to clip wings

Protocol

Fly preparation
Step 1.
Sting 3 holes in a 0.5ml Eppendorf cap and put into 15ml Eppendorf cap with removed lid.

NOTES
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Size of the holes is important: not too small so that enough haemolymph can go through, not too big so that no fat or other dirt is going through.

Fly preparation
Step 2.
Remove the flies' wings, spear the fly's thorax with the peaked stylus.

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

Fly preparation
Step 4.
Centrifuge the 0.5ml Eppendorf cap within the 1.5ml one (1 min, 5000 rpm, at 4°C).

DURATION
00:01:00

Fly preparation
Step 5.

Discard the 0.5ml Eppendorf cap, soak the pellet with a capillary.

Fly preparation

Step 6.

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

Fly preparation

Step 7.

The haemolymph from the capillary can be transferred with the suction cup anywhere.

Enzymatic procedure

Step 8.

Add 19.5µl cold PBS to 0.5µl haemolymph.

Enzymatic procedure

Step 9.

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

Enzymatic procedure

Step 10.

Incubate over night at 37°C.

DURATION

18:00:00

Enzymatic procedure

Step 11.

Add 50µl Tris Buffer.

Enzymatic procedure

Step 12.

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

Enzymatic procedure

Step 13.

Incubate for exactly 30 min at 37°C.

DURATION

00:30:00

Enzymatic procedure

Step 14.

Stop reaction by adding 160µl Sulfuric Acid.

Enzymatic procedure

Step 15.

Measure at 540nm at the (nanoDrop) spectrometer.