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Treatment of D. melanogaster with small molecules in fly food

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We use this protocol and it's working

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Abstract

This protocol describes the preparation of fly food with small molecules to treat flies during their aging. It can be adapted for any treatments. Details for small molecules used can be found in the "Materials" section

Materials

Coenzyme Q10, Merck, Cat# C9538-1G
- 50 mM stock solution in ethanol (99.8%), aliquoted in single-use portions and stored at -20°C
- final concentration of 1 mM with 2% ethanol

Retromer Chaperone, R55, Merck, Cat# 5310840001
- R55 (Calbiochem/Millipore) was dissolved in sterile distilled water as a 12 mM stock solution, aliquoted in single-use portions and stored at -20°C
- final concentration of 12 µM

Levodopa (L-Dopa), Merck, Cat# PHR1271-500MG
- 15.2 mM stock in sterile distilled water on ice and stored at -80°C in single-use aliquots
- 1 mM final concentration

3,4-Dihydroxy-D-phenylalanine (D-Dopa), Merck, Cat# D9378-250MG
- 15.2 mM stock in sterile distilled water on ice and stored at -80°C in single-use aliquots
- 1 mM final concentration

Food preparation

1m

1 warm up the amount of fly food (standard corn meal and molasses food) required (e.g. 50ml for 45 XS vials) in the microwave, stir it in between, start with 1.5 min, mix the food, heat up for 1min again and continue until food has no clumbs anymore

2 prepare XS vials (45 for each condition when 50ml/condition) and label them, place them in plastic boxes

3 measure the amount of food in measuring cylinder and let it cool down in the beaker to around 40 °C (immediately clean all glass ware after usage)

4 add compound/solvent and stir very well (00:01:00), to keep the food liquid put in water bath to maintain the temperature

1m

5 pipette around 1 mL per XS vial with 10 or 25 ml pipette

6 Make sure the food is filling the whole bottom of the vial and shows even surface to avoid flies getting stuck in small wholes when aging

7 store the food in darkness at 4 °C in the cold room and clean up

8 use the food not longer than 2 weeks

fly treatment

9 depending on the experimental condition flies are exposed to the treatment and corresponding control after they are collected (Q10 or R55 treatment) or 10 days prior the experiment (D-/L-DOPA treatment)

10 flies on treatment/control food are kept in a temperature and light-controlled incubator at 25°C and 12-hour light-dark cycle

11 flies are flipped to new food every two to three days

12 On the day of the experiment flies are flipped on fresh treatment/control food at Zeitgeber zero to one