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Real time qPCR (Fly Heads)

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This Protocol describes how to perform RT qPCR with RNA extracted from fly heads.

RNA Extraction

1 Homogenize 6 heads in 50 µL Qiazol, then add 450 µL Qiazol.

2 Spin 00:10:00 at 4 °C at 12,000 x g. 10m

3 Incubate supernatant for 00:05:00 at Room temperature . 5m

4 Add 100 µL chloroform. Vortex, incubate for 00:03:00 at Room temperature , then spin for 00:15:00 at 4 °C at 12,000 x g. 18m

5 Transfer the upper phase to fresh tube, add 250 µL isopropanol, incubate 00:10:00 at Room temperature , spin for 00:10:00 at 4 °C at 12,000 x g. 20m

6 Wash the pellet in 500 µL 75% ethanol (it will be very loose) three times. Do not vortex.

7 Air dry

8 Resuspend in 15 µL RNAse/DNAse-free water.

9 Measure 260/280 and 260/230 on nanodrop.

RT-PCR

10 Use 1 µg RNA to perform RT-PCR reaction.

11 Thermocycler settings 2h 15m

a. 25 °C 00:10:00
b. 37 °C 02:00:00
c. 85 °C 00:05:00
d. 4 °C Hold

qPCR

12 Make master mix with primers, SYBR Green and water.

13 Dilute DNA 1:50 making a 50 µL stock.

14 Add 2 µL DNA to wells with special tips.

15 Add 14 µL master mix to wells with regular tips. Pipette up and down once but do not expel a bubble. Keep master mix on ice the entire time to avoid bubbles.

16 Check for bubbles and run on machine.