NemaMetrix

wMicroTracker Protocol

MEASURING TOXICITY

What you need:

- OP50
- NGM plates
- M9 buffer
- 15 mL tube
- 96-well microplate
- WMicroTracker device

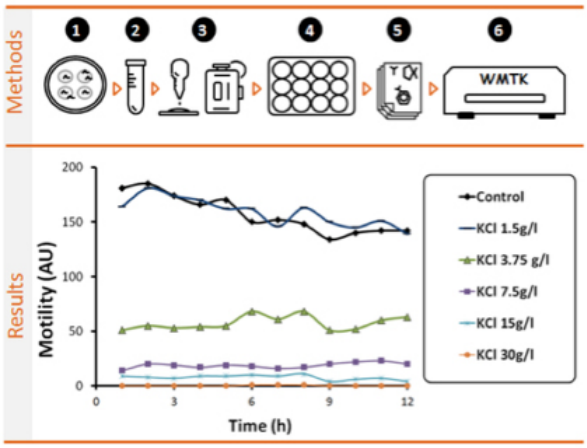
Notes:

- Use M9 buffer supplemented with bacteria OP50 to avoid the static of micro plates.
- Use a saline buffer for tests no longer than 12 hours. The starved worms could affect the results.
- Perform at least three technical replicates and at least two biological replicates.

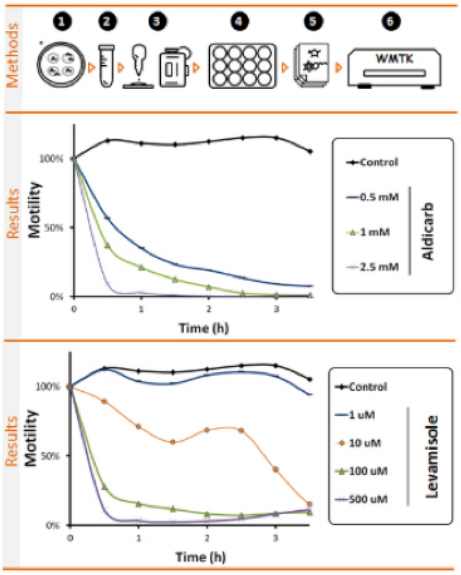
Protocol:

1. Grow synchronized populations of adult worms in seeding NGM plates (OP50).
2. Remove worms from plates using M9 buffer and transfer them in a sterile 15 ml tube.
3. Let the worms settle. Decant the supernatant taking care not to disturb the pellet.
4. Perform a wash with 5 ml of M9 buffer. Briefly shake or invert the tube.
5. Repeat the decantation step. Throw out the supernatant and add 3 ml of M9 buffer.
6. Count number of worms in 10 ul in triplicate and calculate the average.
7. Prepare a suspension to get [5 worms/10 ul]. Adjust volume in M9 buffer supplemented with bacteria OP50 (0.1 OD600 to final concentration).
8. Transfer 90 ul of worm solution to 96-well microplates using multichannel pipette. Let worms rest for 1 hour and measure basal activity for at least 30 minutes using WMicroTracker.
9. Add 10 ul of a 10x concentrated solution of chemicals to test. Include a control without compounds.
10. Register worm activity using wMicroTracker.

Data produced using this protocol:



Measuring KCl Concentration Effect. In this experiment we can observe the effect of increasing concentrations of KCl in young adults N2 C.elegans. The recording of 12h of activity shows an immediate effect of this salt on behavior and deleterious effect at doses higher than 3.75g/l.



Dose Response Of Aldicarb And Levamisole. In these experiments we can observe the kinetic and dose response to two Acetylcholine channels modulators: Aldicarb and Levamisole. In less than one hour a quantitative dose response effect is obtained.