NemaMetrix

wMicroTracker Protocol

MEASURING OXIDATIVE STRESS

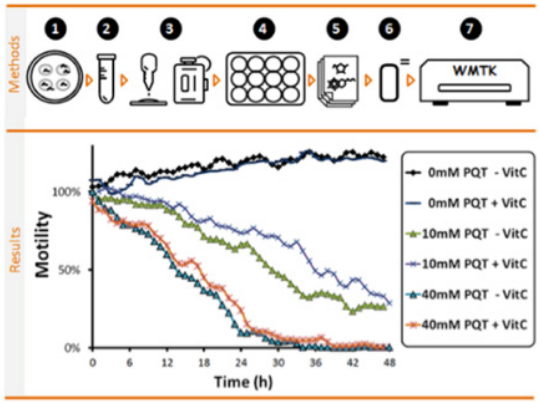
What you need:

- OP50
- NGM plates
- M9 buffer
- Sterile 15 ml tube
- Nutrient medium
- 96-well microplate
- antioxidant molecules
- Paraquat solution with final concentration of 10 mM

Notes:

- An alternative nutrient medium is 3PY medium (3% w/v soy peptone + 3% w/v dry yeast extract + 0.5 mg/ml Hemoglobin in final concentration), Complete S medium supplemented with OP50 1.0 OD600, CeHR or CeMM axenic medium.
- Perform at least three technical replicates and at least two biological replicates.

Data produced using this protocol:



Pharmacological model of oxidative stress using Paraquat

The dynamics of oxidative stress model and a small protective effect of C Vitamin when worms are exposed to 10mM of Paraquat can be observed,

Protocol:

1. Grow synchronized populations of L4 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 nutrient medium (supplemented with 50 µM FuDR + streptomycin 100 µg/ml + kanamycin sulfate 20 µg/ml in final concentration).
6. In triplicate, count the number worms in 10 µl and calculate the average.
7. Prepare a suspension to get [5 worms/10 µl]. Adjust volume in supplemented nutrient medium.
8. Transfer 90 ul of worm solution to 96-well microplates using multichannel pipette.
9. Add 5 ul per well of the potential antioxidant molecules (Include a control without compounds). Incubate at least 4 hours or overnight.
10. Measure basal activity for at least 30 minutes using WMicrotracker.
11. Add 5 ul of a 200mM of Paraquat solution (final concentration of 10 mM).
12. Register worm activity using WMicrotracker.