NemaMetrix

wMicroTracker Protocol

MEASURING HEALTHSPAN

Note: Sterile conditions are required for all the steps below. Use a flow cabinet or a flame.

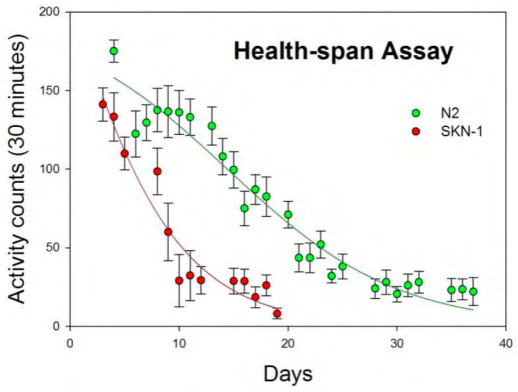
What you need:

i) Master mix solution of feeding bacteria + antibiotics
Prepare 12ml of a master mix containing:

- OP50 (or other food source) (final concentration = 5 mg/ml). See appendix below for details)
- FuDR (final concentration = 100 µM).
- Streptomycin (final concentration = 100 µg/ml)
- Kanamycin sulfate (final concentration = 20 µg/ml)
- Amphotericin B (final concentration = 0.1 µg/ml)
- S-complete buffer to final volume of 15ml

ii) Stock of 6,000 L4 worms:

- Grow 2 plates of synchronized populations of worms in seed NGM plates (OP50) until L4 stage. *We recommend to use at least two 6 cm plates containing a maximum of 5,000 worms each to avoid overcrowding.
- Harvest L4 worms from plates by washing with 4 ml of S-complete solution per plate and transfer them to a sterile 15 ml tube.
- Count number of worms in 10 ul under a microscope (use triplicates). Calculate the average number of worms per 10 µl. Homogenize by shaking the tube manually and discard the appropriate volume to leave 6,000 worms in the tube (this reduces errors, as worms can be lost during the pipetting process) .
- To calculate how many milliliters of solution you need to get 6,000 worms: Use the following formula: [#ml = 10 µl / #worms * 6000 / 1000]



Example data obtain with this protocol:The decreased lifespan of skn-1 mutants can be observed. Samples were recorded for 1 hour once per day, four times per condition.

- Centrifuge the falcon tube at 3000 rpm for 10 minutes to pellet the 6,000 L4 worms and remove the supernatant.

iii) Drug or compound to be tested:
Your drug stock must be concentrated 10x. You will use a final volume of 100 uL for motility measurements.

Basal measurement (optional)

Basal recording is recommended in order to obtains a reference point for motility prior to treatment.

- Stimulate the microplate of worms using high intensity blue light or by shaking the microplate 30 second (100 rpm on an orbital shaker) immediately before basal activity measurement.
- Using the wMicroTracker, record the motility of your worms for 30 minutes. Save this data set as BASAL recording.

Protocol:

Day 1: Passing L4 worms to microplate

1. Add 12 ml of Master mix solution to the 6000 pelleted worms. Shake by hand to homogenize and transfer to a multichannel pipetting receptacle.
2. Transfer 90 ul of worm solution to 96 well microplates (flat bottom) using a multichannel pipette.
3. Cover with the lid and seal with Parafilm to avoid evaporation. Incubate overnight at 20°C.

Note: Water condensation in the plate can be caused by a temperature gradient in the incubator. To prevent this, we recommend that you place another microplate filled water on the shelf above your worms.

Day 2: Drug treatment at 1st day of adult stage

1. On the second day, the animals should be gravid and contain several eggs each.

Drug treatment

* Sterile conditions are needed - Use flow cabinet or flame.
* We recommend that you perform at least 3 technical replicates and at least 2 biological replicates per experiment.
* Include a negative control (buffer without compounds). The Wmicrotracker uses light diffraction. Do not use empty wells as negative control.
* If dissolving the drugs in DMSO: Final concentrations of DMSO should not exceed 0.6%. DMSO concentrations higher than 0.6% shorten C. elegans lifespan and affect worm viability.

- Add 10 ul of the (10X stock) drugs of interest. Note: Calculate the desired concentration in final volume of 100 µl.
- Place the lid and seal the plate with Parafilm.

Day 3 to 21: Measure locomotor activity once a day

Measure activity by following the procedure below (we recommend doing so daily):

- Stimulate the microplate of worms using high-intensity blue light or by shaking the microplate 30 second (100 rpm on an orbital shaker).
- Use the WMicrotracker to measure motility for 30 minutes.Do not remove the lid.
- Return the plates to the 20°C incubator.
- Generate a report file each day and copy/paste the last measure to an integrated datasheet containing all daily data-sets.
- Use appropriate software (i.e. Excel) to generate a line plot to see decay of activity with age.

Day 6, 12 and 18: Fast visual inspection of microplate status, and add more food

A rapid visual inspection (max 5 minutes) of the plate under a microscope is recommended at day 6, 12 and 18 to visualize the health of the worms (i.e. potential contamination, dehydration, larvae hatching). Take note of wells with potential issues in order to discard the corresponding data at time of analysis.

We recommend that you only keep the worms outside of the incubator for a maximum of 5 minutes to avoid major interference with normal culture condition.

After the inspection, follow the procedure below to add food to the wells to prevent starvation and to allows oxygen interchange. Use sterile conditions.

- remove microplate lid
- Add 5 µL of the 100 mg/ml OP50 solution stock to each of the 96 wells. We recommend using a multichannel pipette.
- Place the lid back on the plate and seal with parafilm.
- Shake the microplate for 1 minute to homogenize (100 rpm orbital shaker).

APPENDIX:

Preparation of concentrated bacteria:

- Inoculate 100 ml of LB medium with E.coli OP50 and incubate overnight at 37°C in a bacterial shaker.
- Transfer the OP50 into a sterile, pre-weighted centrifugation tube. Pellet the OP50 by centrifugation for 10 min at 3500 rpm (2200 g) in a table-top centrifuge.
- Discard the supernatant and re-suspend the OP50 pellet in sterile water and re-pellet by centrifugation. Repeat this wash twice.
- After the second wash, carefully remove all the remaining water. No water should be left in the tube. Weigh the centrifugation tube containing the pellet and subtract the weight of the empty centrifugation tube in order to determine the weight of the pellet.
- Thoroughly re-suspend the pellet in S-complete to a concentration of 100 mg/ml. No clumps should be left.
- Store the OP50 solution at 4°C until it is used for the worm culture.