Phylum TECH

Drug
Discovery
Solutions

microtracker

Fast throughput tracking system for small animals

Short Experimental Protocols

SHORT EXPERIMENTAL PROTOCOLS:

C. elegans population size curve in 96 well plate and liquid medium

1) Wash synchronized L4 or adult worms from agar plate with either buffer or water.

2) Generate worm suspensions of increasing number of worms (from 1 to 150 worms per 100ul of medium)

3) Put 100ul of worm suspension into well of a 96 wells plate (flat or U shaped bottoms). Use at least 4 replicate wells per each amount of worms

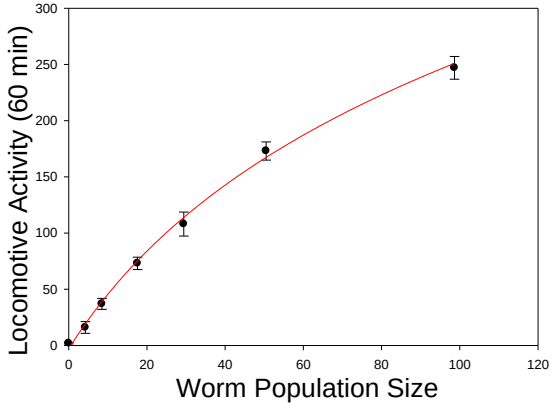
4) Seal the plate with parafilm® and let it settle for 60 min.

5)Setup the replicate groups in the wMicrotracker Software.
6) Place the plate into the wMicrotracker and record activity for 1 hour.

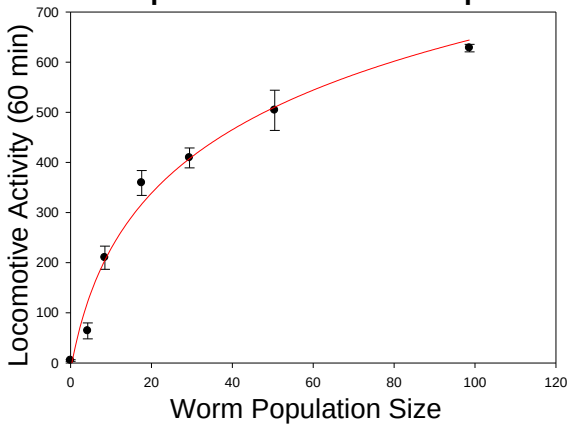
7) Plot activity vs amount of worms on the wells.

Optional: Repeat the experiment with a plate of different well types (U of flat bottom) to compare activity counts and Standard Error.
Optional: Compare the results when generating the report with 30 or 60 minutes time blocks.

96 wells plate with Flat bottom wells



96 wells plate with U-shaped wells



C. elegans Toxicity assay in 96 well plate and liquid medium

1) Wash synchronized L4 or adult worms from agar plate with either buffer or water.

2) Generate a worm suspension of 50 worms per 90ul of medium.

3) Put 90ul of worm suspension into wells of a 96 wells plate.

4) Seal the plate with parafilm® and let it settle for 60 min.

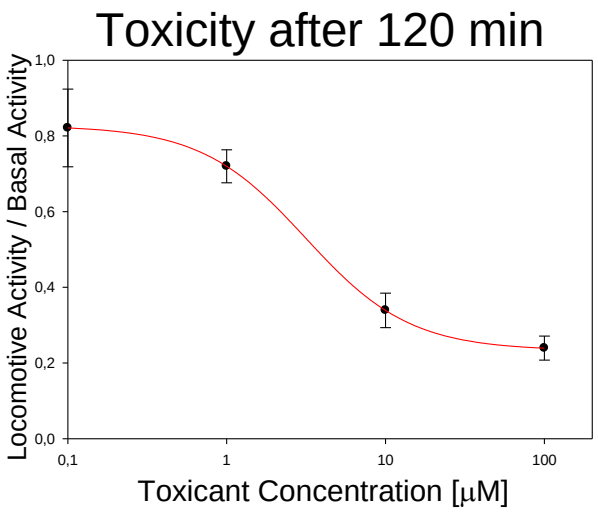
5) Setup the replicate groups (one per toxic concentration) in the wMicrotracker Software.

6) Place the plate into the wMicrotracker and record activity at least for 30min (basal activity).

7) Put 10 ul of toxic solution in at least 4 different concentrations and reseal de plate. Use at least 4 replicate wells per concentration.

8) Place the plate into the WMicrotracker and record activity for 120min. (*you can also measure final point at 60’ or 120’)

9) Plot activity of the last 30min block/basal activity vs toxicant concentration.



C. elegans antioxidant long term experiment (Paraquat + Vitamin C) in 96 well plate and liquid medium

1) Wash synchronized L4 or adult worms from agar plate with either buffer or water.

2) Generate a worm suspension of 50 worms per 90ul of medium.

3) Put 90ul of worm suspension into wells of a 96 wells plate.

4) Seal the plate with parafilm® and let it settle for 60 min.

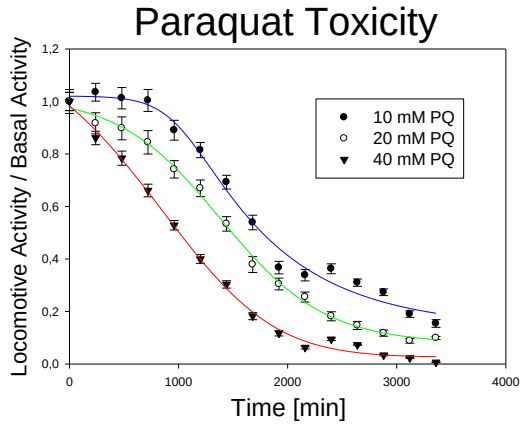
5) Setup the replicate groups (one per toxic concentration) in the wMicrotracker Software.

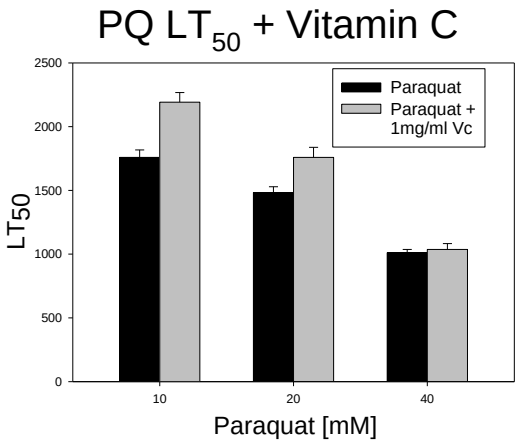
6) Place the plate into the wMicrotracker and record activity at least for 30min (basal activity).

7) Put 10 ul of toxic solution in at least 4 different concentrations and reseal de plate. Use at least 4 replicate wells per concentration.

8) Place the plate into the WMicrotracker and record activity for 120min. (*you can also measure final point at 60' or 120')

9) Plot activity of the last 30min block/basal activity vs toxicant concentration.





Culture medium & buffers

BUFFERS:

K buffer: [1]

32 mM KCl

51 mM NaCl

3PY medium:[2]

3% Yeast extract.

3% Soy peptone.

0.5 mg/ml hemoglobin.

40 uM FUdR, only if you are not using a conditionally sterile strain (I'm starting to use 50 uM that has also been reported).

3PY medium is usually reported with liver extract rather than Hb, we use Hb as a recommendation from Braeckman's lab. First do the 3% YE and 3%SP solution and autoclave it. Separately prepare sterile stock solutions of everything else. To prepare the Hb make up a 50mg/ml solution in 0.1N KOH and autoclave it at 121ºC for no more than 10 min, creating a 100X stock solution.

ANTIBIOTIC MIX: (Reported to doesn't affect lifespan [3])

1000x stock: 200 mg/ml Streptomicyn + 20 mg/ml Kanamicyn.

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References

[1] See: Phillip Williams publications.

[2] See: Bart Braeckman publications.

[3] Nathaniel Szewczyk. Journal of Experimental Biology 209, 4129-4139. 2006

[4] Sergio Simonetta. Journal of Neuroscience Methods. 2007.

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FAQs

¿Is it possible to use another liquid medium?
Yes, 96wells experiments are reported to work with: liquid culture of *E. coli* OP50, CeMM, CeHR (with or without 10% skim milk). It depends of each laboratory preference.
Just be careful with lifespan experiments: it is reported liquid culture in general extend lifespan compared to NGM.

¿Is it possible to use single worm experiments?
Because of the natural variability of *C. elegans'* behavior populational experiments are more robust.
In one of the first papers where we described the method [4], we run experiments with single worms per well in U-bottomed plates, where we kept them for a few weeks.
However, if you like to use a single worm per well you would have to repeat the experiment several times to get reliable data. In this concern, you should have to use at least 16 technical replicates per treatment, U bottom shape microplate and low volume of medium (40ul or less). When using so little medium, evaporation becomes a big deal so don't use the plate lid, instead use a transparent tape. We have used the tape that is used to seal Real Time PCR plates. Then is recommended to pierce two little holes (with a thin needle) in each well to allow gas exchange and prevent water condensation on the top of the wells. With a single worm per well you must place them on each well manually.
Rest of the process should be the same as when using population.

¿How do I get good results if I can't pippete precise number of worms in each well?
Pipetting worms is not as precise to dispensing same number of worms per with a cell sorter. We easily diminish this variability by relativizing mathematically each group of wells to time 0 (we use to call this measurement "BASAL measure").