C. elegans Fast-killing assay (FKA) Protocol

by Erik Andersen March 13, 2008

Plate preparation

1. Prepare FKA plate medium and autoclave

	250 mL	500 mL	1 L
NaCl	2.5 g	5 g	10 g
BactoAgar	4.25 g	8.5 g	17 g
Bactopeptone	2.5 g	5 g	10 g
Sterile water	206 mL	412 mL	824 mL

Autoclave, allow to cool to 55ºC and add the following:

Cholesterol (5 mg/mL in EtOH)	0.25 mL	0.5 mL	1 mL
1 M $CaCl_2$	0.25 mL	0.5 mL	1 mL
1 M $MgSO_4$	0.25 mL	0.5 mL	1 mL
1 M KH_2PO_4	6.25 mL	12.5 mL	25 mL
20 % Glucose	12.5 mL	25 mL	50 mL
1.5 M Sorbitol	25 mL	50 mL	100 mL

2. Pour 4 mL per 35 X 10 mm plate. Flame tops of plates to remove bubbles. Keep plates at 4ºC for a couple of weeks.
3. Inoculate *Pseudomonas arigunosa* (strain PA14) from a freshly streaked plate.
4. Grow for 15 hours at 37ºC.
5. Spot 5 μL onto each plate, spread with a mini-spreader and put plates at 37ºC for 24 hours in a closed box. Be careful not to scratch the tops of the plates.
6. Remove the plates from 37ºC and keep at room temperature for 12 hours. Use plates immediately.

Fast-killing assay

1. Put 25-30 L4 hermaphrodits onto each FKA plate.
2. Put plates at 25ºC.
3. Starting 1 hour later score the number of alive and dead worms.
4. Pick off the dead worms. Dead worms do not move when prodded and sometimes appear clear.
5. Score every hour for the first five hours and then at hour 24.
6. Plot the average percent alive at each time-point for each genotype.