

***C. elegans* Slow-killing assay (SKA) Protocol**

by Erik Andersen December 14, 2007

Assay timing (each step is described below)

- Day 1: Streak out PA14 from frozen stock.
Day 2: Chunk strains for pathogenesis assay
Day 3: Pick 3-5 L4s from chunked strains to new plates, pour SKA plates, inoculate PA14 to LB (at 2 PM)
Day 4: Spot PA14 to center of SKA plate and put plates at 37°C (at 2 PM)
Day 5: Take plates out of 37°C (at 2 PM)
Day 6: Setup 30-50 L4s to each SKA plate
Days 7-11: Score number of dead animals, and for the last time point score the number alive too

Plate preparation

1. Prepare SKA plate medium and autoclave

	250 mL	500 mL	1 L
NaCl	0.75 g	1.5 g	3.0 g
BactoAgar	4.25 g	8.5 g	17 g
Peptone	0.875 g	1.75 g	3.5 g
Sterile water	243.75 mL	487.5 mL	975 mL

Autoclave, allow to cool to 55 °C 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 ₂ PO ₄ (pH 6)	6.25 mL	12.5 mL	25 mL
FUDR, filter sterile in water (100 mg/mL)	125 µL	250 µL	500 µL

2. Pour 4 mL per 35 X 10 mm plate (Falcon #351008). Flame tops of plates to remove bubbles, if needed.
3. Inoculate *Pseudomonas aruginosa* (strain PA14) from a freshly streaked plate.
4. Grow at 37°C overnight for exactly 24 hours.
5. Spot 5 µL of PA14 onto each plate and put the plates at 37°C for 24 hours in a closed box. Be careful not to scratch or puncture the tops of the plates.
6. Remove the plates from 37°C and keep at room temp for exactly 24 hours. Use plates immediately.

Slow-killing Assay

1. Put 30-40 L4 hermaphrodites onto each SKA plate.
2. After all the plates are setup, put the plates at 25°C. The assay time starts now.
3. After 32 hours, score the number of dead worms.
4. Pick off the dead worms. Dead worms do not move when prodded and sometimes appear clear.
5. Score every eight hours, until 96 hours. Then, score every 16 hours. For the 128-hour time point, score the number of dead worms and the number of surviving worms.
6. Plot the percent alive at each time point for each genotype.