

## *C. elegans* Osmotic Stress Resistance Assay

### Reagents Needed:

M9 (common stock)  
500mM NaCl NGM plates (unseeded)

### Procedure:

1. Pour a thin layer of M9 onto an NGM plate containing *non-starved* worms. Gently swirl the plate to dislodge the worms.
2. With a glass pipet, collect the M9 and worms from the plate into microcentrifuge tubes.
3. Centrifuge the tubes at 2000 rpm for 1 minute. A pellet of worms will form.
4. With a glass pipet, place the pellet of worms into the center of a 500mM NaCl NGM plate. *Suck up as little liquid as possible!*
5. Use filter paper to remove any excess liquid from the NGM plate.  
*It is important that you make sure all the excess liquid is gone, or the osmolarity of the plate will change.*
6. Start a timer immediately after all the liquid is gone.
7. Score movement (# moving/total) as needed. Generally, movement is recorded at 3, 5, 7, 9, and 11 minutes. N2 worms should dehydrate and become paralyzed by 7 minutes after the start of the assay. Mutants resistant to osmotic stress will swim longer.

### Recipes:

#### M9 (1L)

\* Common lab stock in worm room.

5.8g Na<sub>2</sub>HPO<sub>4</sub>•7H<sub>2</sub>O

3.0g KH<sub>2</sub>PO<sub>4</sub>

5.0g NaCl

0.25g MgSO<sub>4</sub>•7H<sub>2</sub>O

ddH<sub>2</sub>O to 1L

• Filter (0.22µm) and bottle.

#### 500mM NaCl NGM

Follow the standard NGM protocol, but instead of adding 3.0g/L of

NaCl, add 29.22g/L of NaCl.

Mark plates with a green stripe to distinguish from normal NGM plates.

### Reference:

This assay was developed in the Morimoto Laboratory.

Solomon A, Bandhakavi S, Jabbar S, Shah R, Beitel GJ, Morimoto RI. 2004.

*Caenorhabditis elegans* OSR-1 Regulates Behavioral and Physiological Responses to Hyperosmotic Environments. *Genetics*, 167: 161-170.