Resazurin Assay Preparation & Protocol

Preparation

1. Stock resazurin solution

To make the resazurin stock solution (10 mL) mix the following. We will use this solution for multiple trials.

- 0.5 g resazurin salt
- 10 mL DI water
- 10 µL DMSO

Store in a dark fridge or freezer.

2. Working resazurin solution

To prepare the working solution of resazurin, prepare the following.

This recipe is to make 150 mL of working stock.

- 148 mL seawater (DI water with Instant Ocean adjusted to 23-25 ppt)
- 333 µL resazurin stock solution
- 150 µL DMSO
- 1.5 mL antibiotic solution 100x Penn/Strep & 100x Fungizone - this should be frozen in a dark freezer

Adjust volumes of mix to accommodate number of runs necessary for all individuals undergoing the resazurin trial. Each crab chamber requires 35mL of working solution. 150mL/35mL ≈ 4 crab chamber volumes.

Store at 4°C in dark fridge.

3. Supplies

- 96 well culture plates
- 2 oz condiment cups for crab chambers
- Paper towels or bench paper/pads
- Gloves
- Dissecting microscope
- Spectrophotometer plate reader (Agilent BioTek Synergy HTX with software version 5)

- 5mL transfer pipette with pipette pump
- 200ul pipettor with tips
- 33-35ppt saltwater
- Scale (mg measurements)
- Resazurin working solution

Protocol

1. **Load 35mL of resazurin working solution into crab chambers with transfer pipettor**
2. **Gently pat each crab dry with paper towels and weigh them to the nearest hundredth of a gram**
    a. This will be used to normalize your results across all crabs you test
3. **Carefully place a crab within each chamber and quickly start a timer/stopwatch**
4. **Every 30 minutes, withdraw 200ul from each chamber and place in the wells of the 96 well plate**
    a. Note which wells contain samples from an individual and at what timestep (ex. Well A2: Crab 1, 60min)
5. **At the end of your trials, withdraw crabs from their chambers using gloved hands and rinse them off with 33-35ppt saltwater**
6. **Place crabs back in their tanks, ideally within a partitioned off area to identify them as crabs that have already undergone resazurin analysis**
7. **Run plate in the plate reader at Excitation 530; Emission 590 to obtain fluorescence values**
8. **Divide all fluorescence values by crab weight to normalize for differences in crab size**
9. **Dispose of all resazurin waste in the appropriate chemical waste bottle and thoroughly rinse plates with freshwater**

You TA will run your samples on the plate reader, but if you're interested in how this is done, reference the plate reader SOP.