SOP: Phenotype Assessment of Bivalves (P450)

SOP derived from protocol developed from Counihan et al., 2019 and adapted by Ly Vuthy, UW- Tacoma.

P450 Activity

Prepare solutions prior to homogenizing tissue.

Homogenization buffer: (based on dissected tissue weight)

Obtain an autoclavable media bottle that can hold 150ml

- 2.5ml 1M Hepes
- 0.7305g NaCl
- 50µl 200mM EDTA (Recipe below)
- 100ml deionized H_2O
- Autoclave ***without dithiothreitol*** and store at 4°C
- 0.0001M dithiothreitol – ***add right before use.*** Add 0.1ml of 0.01M dithiothreitol to 10ml of homogenization buffer. (Recipe below)

200mM EDTA:

- 5.84g EDTA
- 100ml deionized water
- Add NaOH to increase pH until salt is in solution
- Autoclave and store at 4°C

0.01M Dithiothreitol: ***make fresh daily in eppendorf tube***

- 0.0015g dithiothreitol
- 1ml deionized water
- Store at 4°C

Microsome buffer:

- 5ml 1M Hepes
- 1.6363g NaCl
- 0.0272g KH_2PO_4
- 200ml deionized water
- Autoclave and store at 4°C

500µM BFC in DMSO stock solution: (Prepare as needed)

- .0016g 7-benzyl-4(trifluoromethyl) coumarin
- 10ml DMSO
- Store at -20°C
- 50µM BFC solution:
- 1ml 500µM BFC in DMSO stock solution
- 9ml phenol red-free DMEM
- Store at -20°C in 5ml aliquots

Stop solution: (Prepare as needed)

- 80ml CH_3CN
- 1.211g Tris base

20ml dH_2O
Store at room temperature

Homogenization Step

Materials:

Razor

Forceps

Microcentrifuge tubes (MCT)

Plastic pestle (must fit MCT)

1. Homogenize half the digestive gland using a razors.
2. Weigh it (between .05g-.07g) and place the tissue into a microcentrifuge tube then add homogenization buffer at a 1:5 weight:volume ratio (remember to add dithiothreitol before use)
   - **Preform volume calculations prior to ensure sufficient buffer is available**
   - ***Smear digestive gland from the scissors onto a slide for MN***
3. Centrifuge at 3,750 rpm (1,500 x g) for 10 minutes at 2°C
4. Transfer supernatant to clean tube
5. Centrifuge supernatant at (9,700 rpm) 10,000 x g for 20 minutes at 2°C
6. Discard supernatant and resuspend pellet in 200ul microsome buffer. Freeze at -80C until use in assay.
   - All samples .05 - .0599 resuspend in 300 µl
   - All samples .06 - .0699 resuspend in 400 µl
   - All samples .07 and above resuspend in 450 µl
7. Measure protein concentration in a Bradford assay
8. Inoculate 50ul of each sample in triplicate into a 96-well black-side, clear-bottom plate

a. Include 50ul of microsome buffer only to be used as a blank

9. Add 50ul of 50µM BFC solution and incubate 4 hours at room temperature
10. Add 40ul of stop solution
11. Read on a plate reader at 410 excitation, 530 emission