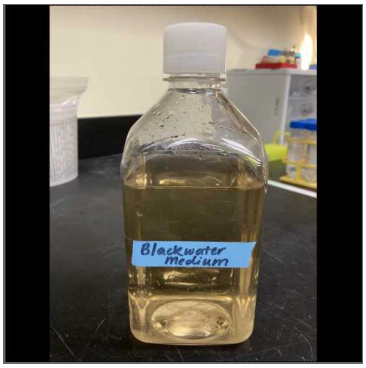


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We are still developing and optimizing this protocol

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# Blackwater medium

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## ABSTRACT

Blackwater medium is a laboratory growth medium for Hydra that replicates the chemical composition of blackwater swamps. This medium has lower low ionic strength than traditional Hydra medium and contains humic acid. Blackwater medium is ideal for Hydra species isolated from blackwater swamps and improves the health of some transgenic Hydra lines that are prone to infection.

## IMAGE ATTRIBUTION

Callen Hyland

## GUIDELINES

Please see Low cost methods for Hydra care for general guidelines for working with Hydra.

MATERIALS

Calcium Chloride Dihydrate **Fisher Scientific Catalog #C79**

Potassium Chloride **Fisher Scientific Catalog #P217**

Sodium Chloride **Fisher Scientific Catalog #S271**

Magnesium Chloride Hexahydrate **Fisher Scientific Catalog #BP214**

Humic Acid Sodium Salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H16752**

- Lab scoops
- Weigh boats or weigh papers
- Electronic balance
- DI water
- 50 mL conical tubes
- Syringe filter or vacuum filter
- Adjustable volume micropipette and tips
- Liter bottles or plastic carboy

## Stock solutions

1 Make stock solutions in 50 mL conical tubes with DI water.

| | A | B | C |
|---|---|---|---|
| | Component | Stock conc. | Weight for 50 mL |
| | Calcium chloride dihydrate (CaCl2*2H2O) | 2.5 M | 18.4 g |
| | Magnesium chloride hexahydrate (MgCl2*6H2O) | 1.0 M | 10.2 g |
| | Sodium chloride (NaCl) | 5.0 M | 14.6 g |
| | Potassium chloride (KCl) | 1.0 M | 3.73 g |
| | Humic acid sodium salt | 20 g/L | 1 g |

Table 1. Stock solutions for blackwater medium.

2 Not all of the humic acid sodium salt will go into solution. Filter the humic acid solution with a syringe filter or vacuum filter to remove undissolved particles.

## Working solution

3 Prepare blackwater medium by diluting stock solutions in DI water. Use a 1 L bottle, or scale up volumes for a large plastic carboy. Mix well before using.

| | A | B | C |
|---|---|---|---|
| | Component | Final conc. | Volume of stock for 1 L |
| | Calcium chloride dihydrate (CaCl2*2H2O) | 0.015 mM | 6.0 µL |
| | Magnesium chloride hexahydrate (MgCl2*6H2O) | 0.0185 mM | 18.4 µL |
| | Sodium chloride (NaCl) | 0.125 mM | 25 µL |
| | Potassium chloride (KCl) | 0.0075 mM | 75 µL |
| | Humic acid sodium salt | 0.01 g/L | 0.5 mL |

Figure 2. Chemical composition of Blackwater medium.