

Chalkley's Medium for Protists



Introduction

Chalkley's Medium is recommended as a growth medium for many fresh water protists. Often boiled wheat, rice or hardboiled egg yolk is added to enhance prey (bacteria) growth.

Materials

Calcium chloride, CaCl_2 , 0.006 g

Potassium chloride, KCl , 0.004 g

Sodium chloride, NaCl , 0.1 g

Rice grains, 8 per liter*

Wheat grains, 8 per liter*

Timothy hay, 7 g per liter*

**Additional materials added as directed.*

Hard-boiled egg yolk, 1 g per liter*

Water, distilled, deionized, or bottled spring

Autoclave

Beaker, 250-mL

Erlenmeyer flask, 2-L

Hot plate or microwave

Foam plug for flask

Safety Precautions

Calcium chloride is slightly toxic. Wear chemical splash goggles, chemical-resistant gloves and a chemical-resistant apron whenever working with chemicals, heat or glassware. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure for *Amoeba* and Amoeba-like organisms

1. Boil 8 rice grains or 7 g of Timothy hay for 1–2 minutes. Allow to cool.
2. In a 2-L Erlenmeyer flask, dissolve each of the three salts into 1 L of distilled, deionized, or bottled spring water.
3. Autoclave the media or sterilize using the Tyndall method (see *Tips*).
4. Add the boiled rice grains or Timothy hay to the media.
5. Allow bacteria to grow on the rice or Timothy hay within the Chalkley's Medium. Typically 1–3 days.
6. Inoculate the Chalkley's Medium with prey for the amoeba such as *Chlorella* or *Chlamydomonas*. These flagellates will feed on the bacteria while the amoeba feed on the small flagellates.
7. Add the *Amoeba* culture to the Chalkley's Medium.

Procedure for *Paramecium* and Paramecium-like organisms

1. Boil 8 rice or wheat grains for 1–2 minutes. Allow to cool.
2. In a 2-L Erlenmeyer flask, dissolve each of the three salts into 1 L of distilled, deionized, or bottled spring water.
3. Autoclave the media or sterilize using the Tyndall method (see *Tips*).
4. Add the boiled rice or wheat to the mixture.
5. Allow bacteria to grow on the rice or wheat within the Chalkley's Medium. Typically 1–3 days.
6. Add the *Paramecium* culture to the Chalkley's Medium.

Procedure for *Vorticella*

1. Hard boil an egg. Allow to cool.
2. In a 2-L Erlenmeyer flask, dissolve each of the three salts into 1 L of distilled, deionized, or bottled spring water.

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- Mix a small amount of the media to 1 g of the hard-boiled egg yolk to make a paste.
- Continue adding small amounts of the media and mixing until the entire 1 L of the salt mixture has been added.
- Autoclave the media or sterilize using the Tyndall method (see *Tips*).
- Allow bacteria to grow on the Chalkley's Medium. Typically 1–3 days.
- Add the *Vorticella* culture to the Chalkley's Medium.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Excess Chalkley's medium may be disposed of down the drain with plenty of excess water according to Flinn Suggested Disposal Method #26b. The wheat, rice, and Timothy hay may be disposed of in the regular trash according to Flinn Suggested Disposal Method #26a. Any unused chemicals may be stored for future use. Cultures should be sterilized before disposal according to Flinn Biological Waste Disposal Method Type I.

Tips

- Non-photosynthetic protists must be supplied with prey in order for the culture to survive. Some prey upon bacteria, others prey upon other protists which feed upon bacteria. No prey is necessary for photosynthetic or symbiotic protists.
- Non-photosynthetic protists thrive in dim to dark lighting conditions. Photosynthetic protists require medium to bright light.
- Maintain cultures at 18–25 °C. Warmer temperatures will cause a bacterial bloom and foul the water due to a lack of dissolved oxygen.
- Replenish evaporated water with bottled spring, deionized or distilled water.
- Do not use iodized sodium chloride.
- Either white or brown rice may be used. Do not use instant rice.
- It may seem odd to autoclave a mixture then wait for bacteria to grow on it. Autoclaving will kill any bacterial contamination from the preparation, including skin bacteria. The culture will acquire airborne environmental bacteria which is the type needed to feed the protists.
- No autoclave? Use the Tyndall method. Bring the solution to near boiling and hold it there for 15 minutes. Repeat the next day and the day after that. Use sterile water to bring the final volume back to 1 L.

Materials for *Chalkley's Medium* are available from Flinn Scientific, Inc.

Catalog No.	Description
C0018	Calcium Chloride, 500 g
P0183	Potassium Chloride, 100 g
S0063	Sodium Chloride, 500 g
FB0540	Timothy Hay, 100 g
FB0541	Wheat Seeds, 100 g

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.