

Proteose medium

This medium is used for the heterotrophic growth of auxenic cultures of *Chlorella* species. It is based on a modification of Bristol's recipe for algae growth from soil or freshwater ponds (Bold, H.C. 1949. The morphology of *Chlamydomonas chlamydogama*, sp. nov. *Bull. Torrey Bot. Club* 76:101-8).

For **1 L of medium** (Final volume)

1. To approximately 900 mL of dH₂O add each of the components (listed below in stock solutions) in the order specified while stirring continuously.
2. Bring total volume to 1.0 L with dH₂O. Check the pH every time and note in lab notebook. pH should be approximately 6.8.
3. Add 1.0 g Bacto-Peptone. (1.0 g/L)
 - *For 1.5% agar medium, add 15 g of agar into the flask; do not mix.
 - *For soft agar (0.8% agar) medium, add 8.0 g of agar into the flask; melt agar in a microwave, swirl to mix, and dispense into 8.0 mL screw-cap tubes.
4. Cover and autoclave medium for 20 minutes at 120° C.
5. Store at refrigerator temperature.

Stock Solutions

Salt	Mass in 100 mL of Distilled water	Volume of Stock in 1.0 L of medium	Millimolarity
NaNO ₃	2.50 g	10.0 mL	2.90 <u>mM</u>
CaCl ₂ ·2H ₂ O	0.25 g	10.0 mL	0.17 <u>mM</u>
MgSO ₄ ·7H ₂ O	0.75 g	10.0 mL	0.30 <u>mM</u>
K ₂ HPO ₄	0.75 g	10.0 mL	0.43 <u>mM</u>
KH ₂ PO ₄	1.75 g	10.0 mL	1.29 <u>mM</u>
NaCl	0.25 g	10.0 mL	0.43 mM