

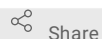


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EMb encystment medium preparation (500 mL)

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1 Works for me



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ABSTRACT

Recipe for EMb encystment medium, which is used to induce synchronous encystment of *Acanthamoeba castellanii* trophozoites. Adapted from Neff protocol.

Neff R, Ray S, Benton W, Wilborn M. Induction of synchronous encystment in *Acanthamoeba* spp. *Methods in cell Physiology* volume 1 (Prescott DM, ed). Academic Press, New York; 1964.

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PROTOCOL CITATION

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KEYWORDS

amoeba, ameba, *Acanthamoeba castellanii*, *Acanthamoeba*, cyst, encystment, encystation, media, medium

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Dry ingredients

- 1 To 500 mL bottle, add:
 - 3.728 g KCl (0.1 M)
 - 1.680 g NaHCO₃ (0.04 M)
 - 0.986 g MgSO₄ × 7H₂O (8 mM)

📏 **0.030 g** $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ (0.4 mM)

📏 **0.017 g** 2-Amino-2-methyl-1,3-propanediol (AMPD, 0.32 mM)

-stir bar

We have found that AMPD is a superior amine buffer to Tris for this medium and maintains a constant pH over time. When Tris is used instead, the pH of the medium steadily decreases to 7 during storage, resulting in poor induction of encystment.

Water

2 Bring volume to 📏 **500 mL** with dH_2O and place on stir plate (unheated).

3 Stir until dissolved.

pH

4 Measure pH using pH meter. Confirm that the pH is between 8 and 8.5--anything in this range works for encystment.

Sterilize

5 Filter sterilize into sterile 500-mL plastic bottle using 0.22 μm filtration system.

Storage in plastic, not glass, is essential to maintaining the pH.

6 Store at 🌡 **Room temperature** .