EMb encystment medium preparation (500 mL)

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1 Works for me Share dx.doi.org/10.17504/protocols.io.bvqcn5sw

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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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Dry ingredients

1 To 500 mL bottle, add:

- **3.728 g** KCl (0.1 M)
- **1.680 g** $NaHCO_3$ (0.04 M)
- **0.986 g** $MgSO_4$ x $7H_2O$ (8 mM)

0.030 g $CaCl_2$ x $2H_2O$ (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** .

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