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Flynn's Encystment Medium preparation (400 mL)

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Protocol status: Working

We use this protocol and it's working

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Abstract

Recipe for FEM (a novel encystment medium) which is used to induce synchronous encystment of *Acanthamoeba castellanii* trophozoites. Results in significantly higher numbers of mature cysts compared to other media (<https://dx.doi.org/10.17504/protocols.io.bvqcn5sw>).

Troubleshooting



Dry ingredients

1 To 500 mL bottle, add:

 4.0664 g $\text{MgCl}_2 \times 6\text{H}_2\text{O}$ (50 mM)

 3.6032 g glucose (50 mM)

 2.9824 g KCl (100 mM)

 1.344 g NaHCO_3 (40 mM)

 0.7888 g $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ (8 mM)

 0.5248 g hydroxyproline (10 mM)

 2 mL 20 mM MnSO_4 (100 μM)

 2 mL 10 mM NiCl_2 (50 μM)

 2 mL 80 mM CaCl_2 (0.4 mM)

 2 mL 64 mM AMPD (2-amino-2-methyl-1,3-propanediol) (0.32 mM)

- stir bar

Dissolve

2 Bring volume to  400 mL with dH_2O and place on stir plate (unheated).

3 Stir until dissolved.

pH

4 Measure pH. Confirm that the pH is between 8 and 8.5 (the optimal range for encystment).

Sterilize

5 Filter sterilize into plastic bottle using 0.22 μm filtration system.

Note

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

6 Store at  4 °C for up to 2 months.

Protocol references

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