

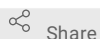


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🌐 PYG medium preparation (2 L)

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1 Works for me



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ABSTRACT

Recipe for PYG rich growth medium for *Acanthamoeba castellanii* trophozoites. Adapted from ATCC Medium: 712 PYG w/ Additives <https://www.atcc.org/~media/F7CFD291FAEC4B26AF234A3A9256C1A3.ashx>

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KEYWORDS

amoeba, ameba, *Acanthamoeba castellanii*, *Acanthamoeba*, medium, media, trophozoite

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Dry ingredients

- 1 To a 2 L bottle, add:
 - ▢ 2 g sodium citrate dihydrate
 - ▢ 2 g yeast extract
 - ▢ 40 g bacto peptone-stir bar

Water

- 2 Bring volume to ▢ 1.8 L with dH₂O (for our reagents, requires adding ▢ 1.68 L dH₂O) and place on stir plate (unheated).

Liquid stock solutions

- 3 Add:
- ▢ **16 mL** 0.05 M $\text{CaCl}_2 \times 2\text{H}_2\text{O}$
 - ▢ **20 mL** 0.4 M $\text{MgSO}_4 \times 7\text{H}_2\text{O}$
 - ▢ **20 mL** 0.25 M Na_2HPO_4 (anhydrous)
 - ▢ **20 mL** 0.25 M KH_2PO_4 (anhydrous)
 - ▢ **20 mL** 0.005 M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6\text{H}_2\text{O}$

All of these nonsterile stock solutions can be made in advance in bulk and stored at room temperature.

0.05 M $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ = ▢ **1.838 g** in ▢ **250 mL** dH_2O

0.4 M $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ = ▢ **24.648 g** in ▢ **250 mL** dH_2O

0.25 M Na_2HPO_4 (anhydrous) = ▢ **8.873 g** in ▢ **250 mL** dH_2O

0.25 M KH_2PO_4 (anhydrous) = ▢ **8.506 g** in ▢ **250 mL** dH_2O

0.005 M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6\text{H}_2\text{O}$ = ▢ **0.196 g** in ▢ **100 mL** dH_2O

- 4 Stir until solution is clear. Check that pH is 6.5 with a pH strip.
- 5 Aseptically add ▢ **100 mL 2 M glucose** to the above medium.

2 M glucose is made in advance, filter sterilized, and stored at 4°C. This is made by **slowly** adding ▢ **180.16 g glucose** to a beaker with approximately ▢ **200 mL dH_2O** while heating and stirring. Once all glucose is dissolved, bring final volume to ▢ **500 mL** with dH_2O and filter sterilize (0.22 μm).

Sterilize

- 6 Filter sterilize into sterile 250-mL glass bottles using 0.22 μm bottle-top filters.
- 7 Store at 🌡 **Room temperature** for up to several months.