PYG medium preparation (2 L)

Carrie A A Flynn[1], Barbara Kazmierczak[1]
[1]Yale University

Jun 14, 2021
1 Works for me Share dx.doi.org/10.17504/protocols.io.bvqbn5sn

Kazmierczak lab

Carrie A Flynn
Yale University

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

DOI

dx.doi.org/10.17504/protocols.io.bvqbn5sn

PROTOCOL CITATION

Carrie A A Flynn, Barbara Kazmierczak 2021. PYG medium preparation (2 L). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvqbn5sn

KEYWORDS

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

CREATED

Jun 10, 2021

LAST MODIFIED

Jun 14, 2021

PROTOCOL INTEGER ID

50659

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 dH_2O (for our reagents, requires adding **1.68 L** dH_2O) and place on stir plate (unheated).

Liquid stock solutions

3 Add:

- **16 mL** 0.05 M $CaCl_2$ x $2H_2O$
- **20 mL** 0.4 M $MgSO_4$ x $7H_2O$
- **20 mL** 0.25 M Na_2HPO_4 (anhydrous)
- **20 mL** 0.25 M KH_2PO_4 (anhydrous)
- **20 mL** 0.005 M $Fe(NH_4)_2(SO_4)_2$ x $6H_2O$

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

- 0.05 M $CaCl_2$ x $2H_2O$ = **1.838 g** in **250 mL** dH_2O
- 0.4 M $MgSO_4$ x $7H_2O$ = **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 $Fe(NH_4)_2(SO_4)_2$ x $6H_2O$ = **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 4ºC. This is made by **slowly** adding **180.16 g glucose** to a beaker with approximately **200 mL dH2O** 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.

Citation: Carrie A A Flynn, Barbara Kazmierczak (06/14/2021). PYG medium preparation (2 L). https://dx.doi.org/10.17504/protocols.io.bvqbn5sn