Nov 06, 2025

Fungal Liquid Carbon Free Media

DOI
https://dx.doi.org/10.17504/protocols.io.14egnrjwml5d/v1

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External link: https://scottc-bio.github.io/CJRS-protocols/

Protocol Citation: Conor James Richard Scott, Davis Roma 2025. Fungal Liquid Carbon Free Media. **protocols.io https://dx.doi.org/10.17504/protocols.io.14egnrjwml5d/v1**

Protocol status: Working
We use this protocol and it's working

Created: October 10, 2025

Last Modified: November 06, 2025

Protocol Integer ID: 229499

Keywords: Microbiology, Minimal, Media, Fungal, Carbon-free, fungal liquid carbon free media, free minimal media for fungal growth, fungal growth, liquid culture, carbon source, basic carbon, added carbon source, base media, different carbon source, free minimal media, agar for solid media, solid media, free media, media, protocol for growth

Disclaimer

> This is not an optimised media for growth. Just a carbon-free base.

Abstract

A simple protocol for the creation of a basic carbon free minimal media for fungal growth. This is not an optimised protocol for growth, it is the base media to which different carbon sources can be added to detect fungal growth and therefore metabolism of added carbon sources. The media can be used for liquid cultures, or combined with agar for solid media.

Guidelines

Can adapt any of the volumes for your requirements. Also can use what other glass vessels for mixing or autoclaving that you want.

Materials

Chemicals

$FeSO_4•H_2O$

EDTA

$ZnSO_4•7H_2O$

H_3BO_3

$MnCl_2•4H_2O$

$CoCl_2•6H_2O$

$CuSO_4•5H_2O$

$(NH_4)_6Mo_7O_{24}•4H_2O$ (Ammonium molybdate)

KOH pellets

1 M KOH

$NaNO_3$

KCl

KH_2PO_4

K_2HPO_4

$MgSO_4•7H_2O$

dH_2O

Glassware

100 mL beaker

250 mL bottle

500 mL beaker

500 mL bottle

Plastic

Sterile 50 mL falcon tubes

Sterile syringes (largest size possible)

0.22 µm pores for filter sterilisation

Equipment

Magnetic stirring equipment (not completely necessary, can stir/shake manually)

pH probe

Laminar flow hood (if unavailable, then can use a bunsen burner and good sterile technique around the sphere of influence)

P1000 + tips (sterile)

Autoclave

Aluminium foil

Troubleshooting

Safety warnings

Always remember when autoclaving to use a larger glass vessel than the volume you want to autoclave to avoid it boiling over.
Always remember to leave the bottle caps loose on duran bottles when autoclaving!
Always wear gloves and eye protection when using 1 M KOH

1000X Hutner's Trace Elements

1 Prepare Solution 1

1.1 Combine the following in a 100 mL glass beaker:
- 1 g $FeSO_4 \cdot H_2O$
- 12.7 g EDTA•2Na

1.2 Add 80 mL dH_2O and stir with a magnetic stirrer until fully dissolved

2 Prepare Solution 2

2.1 Combine the following in a 100 mL glass duran/beaker:
- 4.4 g $ZnSO_4 \cdot 7H_2O$
- 2.2 g H_3BO_3
- 1 g $MnCl_2 \cdot 4H_2O$
- 0.32 g $CoCl_2 \cdot 6H_2O$
- 0.32 g $CuSO_4 \cdot 5H_2O$
- 0.22 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Ammonium molybdate)

2.2 Add 80 mL dH_2O and stir with a magnetic stirrer until fully dissolved

3 Combine solutions 1 and 2 in a 250 mL bottle

4 Adjust the pH with KOH to pH 6.5 by initially using KOH pellets, and then 1 M KOH to gradually increase the pH to the final 6.5

5 Bring the final volume up to 200 mL with dH_2O

6 Cover the bottle with foil and store at 4 °C, during storage the solution will turn from bright green to purple which is normal

20X Fungal Minimal Salts

7 Combine the following in a 500 mL beaker:
- 60 g $NaNO_3$
- 5.2 g KCl
- 8.1 g KH_2PO_4
- 10.5 g K_2HPO_4

8 Add 400 mL dH_2O and stir with a magnetic stirrer until fully dissolved

9 Once dissolved, make up to 500 mL and autoclave

1 M MgSO4

10 Add the following to a 100 mL beaker:
- 12.3 g MgSO4•7H2O

11 Add 40 mL dH2O and stir with a magnetic stirrer until dissolved

12 Once fully dissolved, make up to 50 mL with dH2O

13 Move into a sterilised laminar flow hood and filter sterilise through 0.22 µm pore using a syringe into a sterile falcon tube

Preparing Liquid Carbon Free Media (LCFM)

14 Combine the following in a 500 mL bottle:
- 20 mL 20X fungal minimal salts
- 0.8 mL 1M $MgSO_4$
- 0.4 mL Hutner's trace elements

15 Make up to 400 mL with dH2O

16 Autoclave and allow to cool

17 The media is now ready for liquid cultures