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Bacterial Liquid Carbon Free Media

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

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Protocol status: Working

We use this protocol and it's working

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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 bacterial growth. This is not an optimised protocol for growth, it is the base media to which different carbon sources can be added to detect bacterial 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 you can use whatever other glass vessels for mixing or autoclaving that you prefer or are available.

Materials

Chemicals

$\text{Na}_2\text{HPO}_4 \cdot 5\text{H}_2\text{O}$

KH_2PO_4

NaCl

NH_4Cl

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

$\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

CaCl_2

Agar powder

dH_2O

Glassware

500 mL beaker

500 mL bottle

500 mL bottle (autoclaved)

100 mL beaker

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)

Laminar flow hood (if unavailable, then can use a bunsen burner and good sterile technique)

P1000 pipette + tips (sterile)

P100 + 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 bottles when autoclaving!
Always wear gloves and goggles when handling the 0.1 M HCl.



10X M9 Salts

- 1 Add the following salts to a 500 mL beaker:
 - 27.5 g $\text{Na}_2\text{HPO}_4 \cdot 5\text{H}_2\text{O}$
 - 7.5 g KH_2PO_4
 - 1.25 g NaCl
 - 2.5 g NH_4Cl
- 2 Add 400 mL dH_2O and stir with a magnetic stirrer until dissolved
- 3 Once fully dissolved, make up to 500 mL with dH_2O
- 4 In a laminar flowhood, filter sterilise the solution using a sterile syringe and 0.22 μm pore filters into an autoclaved 500 mL bottle.

1000X Trace metals

- 5 Add the following to a 100 mL beaker:
 - 0.14 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$
 - 0.12 g $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$
 - 0.12 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
 - 0.14 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
- 6 Add 40 mL 0.1 M HCl and stir with a magnetic stirrer until dissolved
- 7 Once fully dissolved, make up to 50 mL with 0.1 M HCl
- 8 Move into a sterilised laminar flow hood and filter sterilise through 0.22 μm pore using a syringe into a sterile falcon tube

1 M MgSO_4

- 9 Add the following to a 100 mL beaker:
 - 12.3 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$



- 10 Add 40 mL dH₂O and stir with a magnetic stirrer until dissolved
- 11 Once fully dissolved, make up to 50 mL with dH₂O
- 12 Move into a sterilised laminar flow hood and filter sterilise through 0.22 µm pore using a syringe into a sterile falcon tube

1M CaCl₂

- 13 Add the following to a 100 mL beaker:
 - 7.35 g CaCl₂
- 14 Add 40 mL dH₂O and stir with a magnetic stirrer until dissolved
- 15 Once fully dissolved, make up to 50 mL with dH₂O
- 16 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)

- 17 Combine the following in a 500 mL bottle:
 - 40 mL 10X M9 Salts
 - 0.4 mL 1000X Trace Metals
- 18 Make up to 400 mL with dH₂O
- 19 Autoclave
- 20 Once cool, in a sterilised laminar flow hood add the following with sterile pipette tips:
 - 0.8 mL 1 M MgSO₄
 - 80 µL 1 M CaCl₂
- 21 Swirl to mix and the media is now ready for use.

