APR 15, 2021

MMH1 Solution

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ABSTRACT

Minimal medium H1 protocol for bacterial competition experiments

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.mknc4ve

Protocol Citation: Erçağ Pinçe 2021. MMH1 Solution. [protocols.io](https://dx.doi.org/10.17504/protocols.io.mknc4ve)
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Protocol status: Working
We use this protocol and it's working

Created: Jan 12, 2018

Last Modified: Apr 15, 2021

PROTOCOL integer ID:
9582

Prepare stock solutions of the salts

1 Prepare Potassium Phosphate:

Name of the chemical	Formula	Brand	Molecular Weight(gr/mol)	Final Concentration	Stock	Factor	Stock in 100mL in gr	
Potassium phosphate(Monobasic)	KH ₂ PO ₄	Sigma-Aldrich	139.06	50 mM	1M	20x	9.5	
Potassium Phosphate(Dibasic)	K ₂ HPO ₄	Sigma-Aldrich	174.18	50 mM	1M	20x	5.25	

- Dissolve the reagents in 80 mL of ddH₂O
- Adjust pH to 7.0
- Make the volume up to 100 mL with ddH₂O
- Filter sterilize and store at room temperature.

Prepare stock solutions of other salts:

Name of the chemical	Formula	Brand	Molecular Weight(g/mol)	Final Concentration	Stock Con.	Factor	Stock in 200 mL in gr	
Ammonium Sulfate	(NH ₄) ₂ SO ₄	Sigma-Aldrich	132.14	7.6mM	760mM	100x	20.09	
Magnesium Sulfate heptahydrate	MgSO ₄	Sigma-Aldrich	246.47	0.5mM	50mM	100x	2.46	
Sodium Chloride	NaCl	Sigma-Aldrich	58.44	67mM	1.34M	20x	15.66	
Iron Sulfate (Cover with foil)	Fe ₂ (SO ₄) ₃	Sigma-Aldrich	399.88	1.25μM	1.25mM	1000x	0.1	

- Mix each compound in 200 mL in separate beakers and stir for ~20mins at room temperature.
- Filter sterilize each solution and store at room temperature.
- **Important Note:** You can either use the stock solution of NaCl [1.34M] or you can directly add it to the final MMH1 mix in its solid form.

Note

The protocol for preparing 1M potassium phosphate was taken from Cold Spring Harbor Protocols

http://cshprotocols.cshlp.org/content/2017/7/pdb.rec090290.full?text_only=true

Prepare MMH1 mix

2 **MMH1:** 1) Add the salts and Thiamine

- 435 mL ddH₂O
- 5 mL Ammonium Sulfate [100x]
- 5 mL Magnesium Sulfate [100x]
- 5 mL Iron(III) Sulfate [100x]
- 25 mL Sodium Chloride [20x]
- 25 mL Potassium Phosphate [20x]
- 50 mg Thiamine Hydrochloride (Sigma-Aldrich)

MMH1[2x]:

- 370 mL ddH₂O
- 10 mL Ammonium Sulfate [100x]
- 10 mL Magnesium Sulfate [100x]
- 10 mL Iron(III) Sulfate [100x]
- 50 mL Sodium Chloride [20x]
- 50 mL Potassium Phosphate [20x]
- 100 mg Thiamine Hydrochloride (Sigma-Aldrich)

2-) Stir mixes at room temperature for ~30mins.

3-) Measure pH and adjust it to 7.0 if necessary.

4-) Filter sterilize and store in the cold room(+4C)