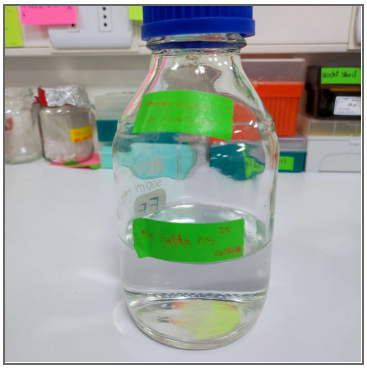

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Protocol status: Working
We use this protocol and it's working

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M9 minimal medium

Isaac Núñez[1], Tamara Matute[1]

[1]LabTecLibres

Laboratorio de Tecnologias Libres

Isaac Núñez
SynBioUC

ABSTRACT

M9 minimal medium is a classical bacterial growth broth (Miller, et al., 1972) characterized by its low autofluorescence.
As it is minimal, it can be supplemented with defined carbon sources or amino acids acord the custom experimental setups.

Recipe

1 This is the recipe for 500 mL total medium.
It is assembled from sterile reagents in sterile conditions (in a laminar flow or flame, and using a sterille glass bottle). They have to be **added in this order** and mixed well after incopore each component.

250 mL Sterile Molecular grade water
100 mL 5X M9 Salt Mix
1 mL $MgSO_4$:$7H_2O$ (1M)
10 mL Glucose (20% w/v) --> 0.4 w/v final concentration in the medium
50 µL $CaCl_2$ (1M)
100 mL Casaminoacids (1%)
complete with ~ 39 mL Sterile Molecular grade water

In case of solid media preparation:
39 mL Sterile Molecular grade water
100 mL 5X M9 Salt Mix
1 mL $MgSO_4$:$7H_2O$ (1M)
10 mL Glucose (20% w/v) --> 0.4 w/v final concentration in the medium
50 µL $CaCl_2$ (1M)
100 mL Casaminoacids (1%)

melt Agar 3% in a microwave or hot bath and complete the media with 250 mL of it.
It should be added quite hot to allow you mix it well and give you enough time to do the plates before it gets solid.

Antibiotic supplementation can be done after adding the Agar.

Plates could be stores at 4°C and darkness for a couple of days but sometimes they form precipitate.

*** **You can replace the carbon source or aminoacids for any other(s) accord your experimental setup** ***

Reagents preparation

2 **5X M9 Salts (600 mL)**

Weight these amounts of each salt and add to a glass bottle:

38.4 g $Na_2HPO_4:7H_2O$
9 g KH_2PO_4
1.5 g NaCl
3 g NH_4Cl

Then, add 600 mL Molecular grade or deionized water and dissolve completely.

Sterilize by autoclaving

3 **$MgSO_4:7H_2O$ (1M) - 20 mL**

Weight 4.930 g of $MgSO_4:7H_2O$
Complete to 20 mL with Molecular grade or deionized water and dissolve completely.

Sterilize by autoclaving

4 **Glucose (20% w/v) - 100 mL**

Weight 20 g of Glucose.
Complete to 100 mL with Molecular grade or deionized water and dissolve completely.

Sterilize by filtering with 0.22 µm syringe filter.

5 **$CaCl_2$ (1M) - 5 mL**

Weight 0.555 g of $CaCl_2$.
Complete to 5 mL with Molecular grade or deionized water and dissolve completely.

Sterilize by filtering with 0.22 µm syringe filter.

6 **Casaminoacids (1%) - 100 mL**

Weight 1 g of Casaminoacids.
Complete to 100 mL with Molecular grade or deionized water and dissolve completely.

Sterilize by autoclaving.

7 **Agar 3% - 500 mL**

Weight 15 g of Agar (molecular biology grade).
Complete to 500 mL with deionized water and dissolve completely.

Sterilize by autoclaving.