

MAR 20, 2023

Standard M9 minimal medium

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ABSTRACT

M9 minimal media is a highly-referenced microbial growth medium used for the cultivation of *E. coli*. This buffered minimal microbial medium contains only salts and nitrogen, so it is traditionally supplemented with glucose, amino acids and vitamins as needed.

M9 Minimal Medium is suitable for non-selective cultivation of *Escherichia coli* strains for cloning, DNA plasmid production and production of recombinant proteins. It is also suitable for selective cultivation when appropriate antibiotics are added. Suitable for non-selective cultivation of *E. coli* strains for cloning, DNA plasmid production and production of recombinant proteins. Also suitable for selective cultivation when appropriate antibiotics are added.

GUIDELINES

Prepare enough for the necessary number of experiments to perform in order to prevent batch-to-batch variation between experiments. Input chemicals should be of a certain quality in order to prevent contaminants that can impact microbial growth and selection.

MATERIALS

Magnetic stirrer, autoclave, scale, tubes, flask

SAFETY WARNINGS



Be sure to wear appropriate PPE when working with antibiotics and chemicals. Take care when working with hot flasks and tubes.

OPEN ACCESS

Protocol Citation: Andreas Sagen 2023. Standard M9 minimal medium.

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Protocol status: In development
We are still developing and optimizing this protocol

Created: Mar 17, 2023

Last Modified: Mar 20, 2023

PROTOCOL integer ID:
79007

Keywords: M9 minimal medium, *e. coli*, *Escherichia coli*, bacteria, autotrophic medium

100 mL 10x Ammonium chloride solution

1h


1 In a bottle, fill with 400 mL deionized water

5m

2 Measure and add: 15m
 50 g Ammonium chloride

Materials:

 Ammonium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4514**


3 Adjust pH to  7.4 using hydrogen chloride or sodium hydroxide 5m

4 Fill bottle to  500 mL with deionized water 2m

5 Sterilize with a 0.2-micron filter 20m

1 000 mL 10x M9 salt solution

2h

6 In a bottle, fill with  800 mL deionized water 5m


7 Measure and add: 15m
 60 g Sodium phosphate (dibasic)
 30 g Monopotassium phosphate
 5 g Sodium chloride







Materials:

 Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S0876**

 Potassium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5379**







 Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S9625**

8 Adjust pH to  7.4 using hydrogen chloride or sodium hydroxide 5m

- 9 Fill bottle to  900 mL with deionized water 2m
- 10 Add  100 mL 10x Ammonium chloride solution 1m
- 11 Autoclave solution at  121 °C for  00:45:00 45m
- 12 Aliquot into  100 mL units and store at  4 °C 30m


500 mL 20% Glucose solution

1h 30m

- 13 In a bottle, fill with  400 mL deionized water 5m
- 14 Measure and add:
 100 g Glucose 15m
- Materials:
 Glucose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G7021**
- 15 Fill bottle to  500 mL with deionized water 5m
- 16 Autoclave solution at  121 °C for  00:40:00 40m

100 mL 1 M Magnesium sulfate solution

45m

17 In a bottle, fill with  80 mL deionized water 2m


18 Measure and add: 5m

 12.04 g Magnesium sulfate

Materials:


 Magnesium sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M7506**

19 Fill bottle to  100 mL with deionized water 1m


20 Autoclave solution at  121 °C for  00:25:00 25m

100 mL 0.5 M Calcium chloride solution

45m


21 In a bottle, fill with  80 mL deionized water 2m

22 Measure and add: 10m

 7.35 g Calcium chloride

Materials:

 Calcium chloride dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C3881**

23 Fill bottle to  100 mL with deionized water 1m

24 Autoclave solution at  121 °C for  00:25:00


25m

100 mL 1 mg/mL Thiamine hydrochloride

45m

25 In a bottle, fill with  80 mL deionized water

2m

26 Measure and add:
 100 mg Calcium chloride

10m

Materials:

 Thiamine hydrochloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #T1270

27 Fill bottle to  100 mL with deionized water

1m

28 Sterilize with a 0.2-micron filter

2m

29 Aliquot into  1 mL aliquots, and store at  -20 °C

30m

500 mL M9 minimal medium










20m

30 In a bottle, fill with  400 mL sterile water


5m

31 Add:

5m


-  7.5 g Agar (Optional)
-  50 mL 10x M9 salt solution
-  10 mL Glucose solution (20%)
-  1000 μ L Magnesium sulfate solution ( 1 Molarity (M))
-  100 μ L Calcium chloride solution ( 0.5 Molarity (M))
-  500 μ L Thiamine hydrochloride solution ( 1 mg/mL)

Note



If using agar, autoclave solution with agar only, then cool to approx.  50 °C , then add the other components. Keep hot enough for the medium to stay liquid, then pour immediately.

Materials:

 Agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1296**

32 Adjust pH to  7.4 using hydrogen chloride or sodium hydroxide

5m

33 Fill bottle to  500 mL with sterile water and store at  4 °C

5m

Note

While it shouldn't be necessary to sterilize if working aseptically, it is possible to sterilize using a 0.2-micron filter. DO NOT AUTOCLAVE!

Note

Supplement medium with antibiotics, etc. as necessary