

M9 Supplemented Media

Introduction

Prof. Lucks lab's protocol for preparing supplemented M9 media

Storage:

* Media should be stored at **4°C**, wrapped in foil and stored away from light

* Media has short shelf life of ~1 week. Check the media for precipitation before using. **For best results, mix the media the day it is to be used**

* Individual media components can be stored long term at **4°C**, except for **thiamine hydrochloride, which should be made fresh and stored away from light**

Materials

- › 1x M9 salts
- › 1 mM thiamine hydrochloride
- › 0.4% glycerol
- › 0.2% casamino acids
- › 2 mM MgSO₄
- › 0.1 mM CaCl₂
- ›

Procedure

1. 5x M9 Minimal Salts

11.3 g M9 minimal salts
300 mL ddH₂O
Autoclave to sterilize

2. 10 mg/mL thiamine hydrochloride

450 mg thiamine hydrochloride
45 mL ddH₂O
0.22 µM filter sterilize

Storage:

Aliquot into 5mL tubes
Freeze with aluminum foil at 20°C
For fridge-stored (+4°C), good for 2 weeks

3. 40% glycerol

80 mL glycerol
120 mL ddH₂O
Autoclave to sterilize

4. 10% casamino acids

10 g Bacto casamino acids
100 mL ddH₂O

0.22 uM filter sterilize

Storage:

Aliquot into 10mL tubes

Freeze at 20°C

5. 1M MgSO₄

6.02 g MgSO₄

50 mL ddH₂O

Autoclave to sterilize

6. 1M CaCl₂

5.55 g CaCl₂

50 mL ddH₂O

Autoclave to sterilize

7. ddH₂O

Autoclave to sterilize

Recipe for mixing:

MIX UNDER FLAME

Add CaCl₂ LAST; goes back into solution once mixed

200 mL M9		
	A	B
1	Volume	Component
2	40 mL	5x M9 minimal salts
3	6.8 mL	10 mg/mL thiamine hydrochloride
4	2 mL	40% glycerol
5	4 mL	10% casamino acids
6	400 uL	1M MgSO ₄
7	20 uL	1M CaCl ₂
8	Fill to 200mL (~147 mL)	Sterilized ddH ₂ O

50 mL

	A	B
1	Volume	Component
2	10 mL	5x M9 minimal salts
3	1.7 mL	10 mg/mL thiamine hydrochloride
4	500 uL	40% glycerol
5	1 mL	10% casamino acids
6	100 uL	1M MgSO ₄
7	5 uL	1M CaCl ₂
8	Fill to 50 mL	Sterilized ddH ₂ O

65 mL

	A	B	C	D
1	Volume	Component		
2	13 mL	5x M9 minimal salts		
3	2.21 mL	10 mg/mL thiamine hydrochloride		
4	650 uL	40% glycerol		
5	1.3 mL	10% casamino acids		
6	130 uL	1M MgSO ₄		
7	6.5 uL	1M CaCl ₂		
8	Fill to 65	Sterilized ddH ₂ O	~47.7 mL	