

# Media

LB



SIS

M9

- M9

## Make M9 salts

To make M9 Salts aliquot 800ml H<sub>2</sub>O and add

- 64g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O
- 15g KH<sub>2</sub>PO<sub>4</sub>
- 2.5g NaCl
- 5.0g NH<sub>4</sub>Cl
- Stir until dissolved
- Adjust to 1000ml with distilled H<sub>2</sub>O
- Sterilize by autoclaving

Measure ~700ml of distilled H<sub>2</sub>O (sterile)

Add 200ml of M9 salts

Add 2ml of 1M MgSO<sub>4</sub> (sterile)

Add 20 ml of 20% glucose (or other carbon source)

Add 100ul of 1M CaCl<sub>2</sub> (sterile)

Adjust to 1000ml with distilled H<sub>2</sub>O

- LB (1000 mL):
- Measure out the following:
  - 10 g tryptone
  - 5 g yeast extract
  - 10 g NaCl
- Suspend the solids in ~800 ml of distilled or deionized water.
- Add further distilled or deionized water, in a measuring cylinder to ensure accuracy, to make a total of 1 liter.
- Autoclave at 121 °C.
- After cooling, swirl the flask to ensure mixing, and the LB is ready for use.



- **Sistrom's Minimal Medium A: for the Growth of Rhodobacter sphaeroides**

Reference: Sistrom, W. R. 1962 . The kinetics of the synthesis of photopigments in Rhodopseudomonas sphaeroides. J. Gen. Microbiol. 28:607-616.

- Preparation of 1 liter of 10X medium

$K_2HPO_4$ or $KH_2PO_4$	34.8 g
$(NH_4)_2SO_4$ or $NH_4Cl$	5.0 g 1.95 g
Succinic acid	40.0 g
L-Glutamic acid	1.0 g
L-Aspartic acid	0.4 g
NaCl	5.0 g
Nitrilotriacetic acid	2.0 g
$MgSO_4 \cdot 7H_2O$ or $MgCl_2 \cdot 6H_2O$	3.0 g 2.44 g
$CaCl_2 \cdot 2H_2O$	0.334 g
$FeSO_4 \cdot 7H_2O$	0.020 g
$(NH_4)_6Mo_7O_{24}$ (1% solution)	0.2 ml
Trace Elements Solution	1 ml
Vitamins Solution	1 ml

Trace Elements Solution (100 ml)

EDTA	1.765 g
$ZnSO_4 \cdot 7H_2O$	10.95 g
$FeSO_4 \cdot 7H_2O$	5.0 g
$MnSO_4 \cdot H_2O$	1.54 g
$CuSO_4 \cdot 5H_2O$	0.392 g
$Co(NO_3)_2 \cdot 6H_2O$	0.248 g
$H_3BO_3$	0.114 g

- 2. Adjust to Ph 7.0 with KOH.
- 3. Add 2 g casamino acids per liter 1X Sistrom's (optional).
- 4. Bring volume to 1 liter.
- 5. For Sistrom's agar add agar to 1.5%.
- 6. Sterilize by autoclaving.
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Notes:

- \* In order to alleviate the problem of calcium phosphate precipitation during autoclaving, the concentration of succinic acid has been doubled and the calcium chloride concentration has been reduced by one half (see reference). No differences in growth of *R. sphaeroides* have been observed with these changes in the medium.
- \* For standard growth, glutamic acid, aspartic acid, and/or casamino acids are not required.
- For low  $SO_4$  medium (eg, when labeling with  $H_2^{35}SO_4$ ) use chloride salts instead of sulfate salts.
- \* For de Cohen-Bazire medium add 1g of yeast extract per liter of sis medium