M9 Minimal Medium

This protocol describes the preparation of M9, a defined liquid growth medium.

Materials

- Milli-Q water
- 5X M9 minimal salts
- 1 M $MgSO_4$
- 1 M $CaCl_2$
- 100X trace elements
- Carbon source of choice (e.g. 20% w/v glucose)

Equipment

- Pipette boy
- Serological pipettes
- Sterile container (falcon tube or glass bottle)
- Pipettes and sterile pipette tips (0.5–10, 2–20, 20–200, 100–1000 µL)

All stock solutions must be sterile, and all steps are carried out aseptically.

Volumes Required

Component	per 100 mL	per 1 L
5X M9 minimal salts	20 mL	200 mL
1 M $MgSO_4$	0.2 mL	2 mL
1 M $CaCl_2$	10 µL	0.1 mL
100X trace elements	1 mL	10 mL
20% w/v glucose*	2 mL	20 mL
Milli-Q water	to 100 mL	to 1 L

* Or another carbon source; 2 mL of 20% w/v glucose per 100 mL gives 0.4% final

Protocol

1. Work in a flow cabinet or next to a Bunsen burner, and use a sterile container of at least the final volume.
2. Add Milli-Q water to roughly 70% of the final volume.
3. Add the 5X M9 salts and swirl to mix.
4. Add the $MgSO_4$, then the $CaCl_2$ dropwise while swirling. Adding them quickly, or to undiluted salts, causes a white phosphate precipitate.
5. Add the trace elements and the carbon source.
6. Top up to the final volume with Milli-Q water and mix by inversion.
7. Add antibiotic if required.
8. Check that the medium is clear before use. Store at 4 ℃.

M9 Minimal Agar Plates

This protocol describes the preparation of M9 agar plates, a defined solid growth medium.

Materials

- Milli-Q water
- Agar (15 g/L final)
- 5X M9 minimal salts
- 1 M $MgSO_4$
- 1 M $CaCl_2$
- 100X trace elements
- Carbon source of choice (e.g. 20% w/v glucose)
- Antibiotic (as needed)

Equipment

- Autoclave
- Schott bottle (at least twice the medium volume)
- Water bath set to 55 °C
- Petri dishes (90 mm)
- Pipette boy and serological pipettes
- Pipettes and sterile pipette tips (0.5–10, 2–20, 20–200, 100–1000 µL)
- Bunsen burner or flow cabinet

All stock solutions must be sterile, and all additions are made aseptically.

Volumes Required

Component	per 500 mL	per 1 L
Agar	7.5 g	15 g
Milli-Q water (autoclaved with the agar)	400 mL	800 mL
5X M9 minimal salts	100 mL	200 mL
1 M $MgSO_4$	1 mL	2 mL
1 M $CaCl_2$	50 µL	0.1 mL
100X trace elements	5 mL	10 mL
20% w/v glucose*	10 mL	20 mL

* Or another carbon source; this gives 0.4% final. 500 mL pours about 20 plates

Protocol

1. Suspend the agar in the Milli-Q water in a Schott bottle of at least twice the final volume, and autoclave at 121 °C for 20 min.
2. Cool the molten agar to 55 °C in the water bath. Hot agar precipitates the salts, and pouring above 60 °C warps the plates.
3. Pre-warm the 5X M9 salts, add them to the agar and swirl without making bubbles.
4. Add the $MgSO_4$, then the $CaCl_2$ dropwise while swirling, then the trace elements, the carbon source and any antibiotic.
5. Pour 20–25 mL per 90 mm plate next to a flame, and flame off any bubbles.
6. Let the plates set for 30 min with the lids on, then dry them and store them inverted at 4 °C in a sealed bag.

Yeast Extract Peptone Dextrose (YPD) Medium

This protocol describes the preparation of 1 L of liquid yeast extract peptone dextrose (YPD) medium, used for bacterial cellulose production

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Materials

- Demi water
- Yeast extract
- Bacterial peptone
- Glucose

Equipment

- Schott bottles, 1 L and 100 mL
- Analytical balance
- pH meter
- Hot plate
- Autoclave
- Flow cabinet

Quantities Required

Component	Amount
Demi water, in the 1 L bottle	950 mL
Yeast extract	10 g
Bacterial peptone	10 g
Glucose*, in the 100 mL bottle	20 g
Demi water	50 mL

* Or another carbon source

Protocol

In the 1 L Schott bottle:

1. Fill the bottle with 950 mL demi water.
2. Add the yeast extract and bacterial peptone, and swirl until they are thoroughly combined with the water.
3. Measure the pH of the resulting solution and, if needed, adjust it to pH 6.5.

In the 100 mL Schott bottle:

4. Mix 20 g glucose with 50 mL demi water.
5. Swirl until combined, placing the bottle on a hot plate to dissolve the glucose fully.

Sterilisation:

6. Autoclave both Schott bottles with their caps loose.
7. Once cooled, add the glucose solution to the 1 L medium in the flow cabinet to avoid contamination.

Uracil Dropout Yeast Medium (SD–Ura)

This protocol describes the preparation of a defined liquid yeast medium lacking uracil, used to select for plasmids carrying a *URA3* marker.

Materials

- Deionised water
- Yeast Nitrogen Base without amino acids
- Glucose
- Y1501 amino acid dropout supplement (without uracil)

Equipment

- Balance and weighing boats
- Beaker and magnetic stirrer with stirring bar
- Measuring cylinder
- 0.22 µm bottle-top filter or syringe filter
- Sterile bottle for the filtered medium
- Pipettes and sterile pipette tips

The medium is filter-sterilised, not autoclaved.

Quantities Required

Component	per 1 L	per 100 mL
Yeast Nitrogen Base w/o amino acids	6.8 g	0.68 g
Glucose	20 g	2 g
Y1501 dropout supplement (–Ura)	1.92 g	0.192 g
Deionised water	to 1 L	to 100 mL

Final concentrations: 0.68% w/v YNB, 2% w/v glucose

Protocol

1. Weigh out the Yeast Nitrogen Base, glucose and Y1501 dropout supplement.
2. Add them to roughly 80% of the final volume of deionised water in a beaker with a stirring bar.
3. Stir until everything has fully dissolved. The dropout powder is slow to go into solution; do not heat the medium.
4. Top up to the final volume with deionised water and mix.
5. Filter-sterilise through a 0.22 µm filter into a sterile bottle, working next to a flame or in a flow cabinet.
6. Label the bottle and store at 4 °C.