

Andersen Lab Recipes

M9 Buffer

Mix the following:

Reagent	Amount Needed to make: 1L of 1X stock	Amount Needed to make: 3L of 1X stock
KH ₂ PO ₄	3 g	9 g
Na ₂ HPO ₄	6 g	18 g
NaCl	5 g	15 g
dH ₂ O	up to 1L	up to 3 L

To make 3 L of 1X Stock

1. First measure all reagents into 2.5 L distilled water in a 4 L beaker and fully dissolve. Then bring volume to total of 3 L in a graduated cylinder.
2. Filter Sterilize using the vacuum filtration systems into 500 mL bottles (6 bottles total) (Thermo- 0.45 uM, Rapid Flow PES)
3. Autoclave on liquid cycle, 30 minutes (Make sure to loosen the cap only a little, to maintain final volume).
4. When the M9 is cool, add: MgSO₄, filter-sterilized; add 0.5 ml for 500 mL of M9

4 L of 4X M9 Stock

1. Mix the following:

Reagent	Amount Needed: 4X Stock to make 4 L
KH ₂ PO ₄	48 g
Na ₂ HPO ₄	96 g
NaCl	80 g
dH ₂ O	up to 4 L

About 32 L of 1X M9 is required per week during maximum Sorter use. Make a minimum Total of 12 L of 4X Stock at a time and use one filter. This batch of 4X Stock should last at least 1.5 weeks. Also be sure to have 1 L of 1X M9 in 250 ml bottles on the shelf at all times.

- a. Measure all reagents into **3.0 L of distilled water** in a 4 L beaker and fully dissolve. Then bring the volume to total of 4 L in a graduated cylinder, and mix thoroughly.
2. Transfer the 4X stock to 500 mL bottles using filter sterilization, measuring exactly. Filter Sterilize using the vacuum filtration systems (Thermo 0.45 uM, Rapid Flow PES).
3. Repeat step 1 to make a total of 12 L of 4X Stock. Make sure you have 24 empty 500 ml bottles available.
4. To complement the 4X M9 stocks, prepare 1500 ml of sterile distilled water, premeasured into 2 L glass bottles.
5. Autoclave filtered M9 and 1500 mL water on the liquid cycle, 30 minutes sterilization.
 - a. Make sure to loosen the cap only a little on glass bottles, to maintain final volume.
6. When the M9 is cool, add:
 - a. MgSO₄, (1 M, filter sterilized): 2 ml for 500 ml of 4X Stock
7. To make a 1X M9 working stock of 2 L, mix one of the bottles of 500 ml of 4X Stock with the bottle of 1500 ml sterile water.

Potassium Phosphate Buffer, 1M pH6

Mix the following:

Reagent	Amount Needed
KH ₂ PO ₄	136.1 g
KOH	17.99 g
dH ₂ O	up to 1 L

1. Filter Sterilize into a 1L Sterile Bottle using the vacuum filtration systems (EMD- 0.22 µM, Express-Plus).
2. Store in 1 L aliquots.

S Basal

Mix the following:

Reagent	Amount Needed
NaCl, 5 M	20 ml
Potassium Phosphate Buffer, 1 M pH 6	50 ml
Cholesterol, 5 mg/ml in ethanol	1 ml
dH ₂ O	930 mL

- Autoclave on liquid cycle for 30 minutes. Note that the solution will be cloudy

S Medium

1. Make each component below according to the recipe.
2. Using sterile technique, mix the following:

Reagent	Amount Needed
5 M NaCl	40
Potassium Citrate, 1 M pH 6	20 ml
Trace Metals Solution	20 ml
CaCl ₂ , 1 M	6 ml
MgSO ₄ , 1 M	6 ml
Phosphate Buffer, 1 M pH6	20 ml
dH ₂ O	1886

3. Filter sterilize using a 0.22 µm filter in 499.5 ml aliquots.
4. **Using good sterile technique**, add 0.5 ml of cholesterol (5 mg/ml on EtOH) to each aliquot.

Trace Metals Solution

1. Dissolve 3.06 g Trace Metals Mix (US Biological, cat # N1010) into 1 L of dH₂O.
 - a. Stir with heat until completely solubilized.
2. Aliquot 100 ml into amber PDPE bottles.
3. Autoclave on liquid cycle for 30 minutes, using the large autoclave on the left.
 - a. Be careful to just place the cap on top of the bottle
 - i. If you screw the cap, the bottle could deform during autoclaving

Potassium Citrate, 1 M pH 6

1. Mix the following:

Reagent	Amount Needed
Citric Acid Monohydrate	20 g
Tri-potassium Citrate Monohydrate	293.5 g
dH ₂ O	up to 1 L

2. Autoclave on liquid cycle for 30 minutes.

Freezing Solution

1. Mix the following — dissolve the Glycerol in water first, then mix in other reagents.

Reagent	Amount Needed
Potassium phosphate buffer, 1 M pH 6	100 ml
NaCl, 5 M	40 ml
Glycerol, 100%	600 ml
dH ₂ O	up to 2 L

2. Divide into 200 ml aliquots in 250 mL glass square bottles.
3. Autoclave on liquid cycle for 30 minutes.
4. IMMEDIATELY BEFORE USE: Add MgSO₄ to a final concentration of 0.3 mM (e.g. for 200 ml of freezing solution, add 60 µl of 1 M MgSO₄).

Bleach Solution

Reagent	Amount Needed for 10 ml	Amount Needed for 200 ml
NaOCl (from Fisher, cat #SS290-1)	2 ml	40 ml
NaOH Pellets*	0.2 g	4 g
dH ₂ O	up to 10 ml	up to 200 ml

* **NOTE:** If using a 10 M NaOH solution, add 0.5 ml to 10 ml Total Bleach Solution.

Store at 4°C

Horvitz Super Broth

1. Mix the following in a 6 L Erlenmeyer flask:

Reagent	Amount Needed
Tryptone	30 g
yeast extract	60 g
Glycerol, 100%	10 ml
dH ₂ O	2.25 L

- a. Be careful to be accurate when measuring the glycerol
 - b. Add glycerol after the water.
2. Pipette broth solution up and down 2-3 times to remove glycerol from the inside of the pipetter.
 3. Autoclave on liquid cycle for 30 minutes.
 4. Once the media is cool, add 250 ml of sterile super broth potassium phosphate buffer (0.17M KH₂PO₄, 0.72M K₂HPO₄) for a total of 2.5 L.

5X Horvitz Super Broth

1. Mix the following:

Reagent	Amount Needed
Tryptone	30 g
yeast extract	60 g
Glycerol, 100%	10 ml
dH ₂ O	up to 450 ml

- a. Be careful to be accurate when measuring the glycerol.
 - b. Add glycerol after the water.
2. Pipette broth solution up and down 2-3 times to remove glycerol from the inside of the pipetter.
 3. Autoclave on liquid cycle for 30 minutes.
 4. Once the media is cool, add 50 ml of sterile 5X super broth potassium phosphate buffer (0.85M KH₂PO₄, 3.56M K₂HPO₄).

Super Broth Potassium Phosphate Buffer

1. Mix the following:

Reagent	Amount Needed
KH ₂ PO ₄	46.2 g
K ₂ HPO ₄	250.8 g
dH ₂ O	up to 2 L

2. Filter Sterilize into a 500 mL Sterile Bottle using the vacuum filtration systems (EMD- 0.22 µM, Express-Plus). Store in 500 ml aliquots.

5X Super Broth Potassium Phosphate Buffer

1. Mix the following:

Reagent	Amount Needed
KH ₂ PO ₄	5.78 g
K ₂ HPO ₄	31.35 g
dH ₂ O	up to 50 ml

2. Autoclave on liquid cycle for 30 minutes.

50X TAE

1. Mix the following:

Reagent	Amount Needed
Tris Base	242 g
EDTA, 0.5 M pH 8	100 ml
Glacial Acetic Acid	57.1 ml
dH ₂ O	up to 1 L

- a. Be very careful when pipetting the glacial acetic acid, as it is very caustic.
- b. Use a GLASS pipette

EDTA, 0.5 M

1. Mix the following:

Reagent	Amount Needed
EDTA, disodium salt	93 g
NaOH pellets	~10 g
dH ₂ O	up to 500 mL

2. Add EDTA powder to ~450 ml of dH₂O with stirring and monitoring the pH.
3. Slowly add the NaOH pellets until the solution is pH 8.
4. Bring the volume up to 500 ml.
5. Filter sterilize with 0.2 μ m filter.

LB Miller Broth

1. Mix the following:

Reagent	Amount Needed
Tryptone	10 g
yeast extract	5 g
NaCl	10 g
dH ₂ O	up to 1 L

2. Autoclave on liquid cycle for 30 minutes.
3. Once the media is cooled after autoclaving, you can add antibiotics (if needed):

Antibiotic	Stock	Dilution	Final Concentration	Volume Added to 500 ml of Media
Ampicillin	100 mg/ml	1:1000	100 µg/ml	0.5 ml
Kanamycin	50 mg/ml	1:1000	50 µg/ml	0.5 ml
Chloramphenicol	50 mg/ml	1:3333	15 µg/ml	0.15 ml

4. Store media with antibiotics at 4°C.

LB Miller Agar

1. Mix the following:

Reagent	Amount Needed
Tryptone	10 g
yeast extract	5 g
NaCl	10 g
Agar	15 g
dH ₂ O	up to 1 L

2. Autoclave on liquid cycle for 30 minutes.
3. Once the media is out of the autoclave, place it in a 55°C oven for 1 hour.
4. After the media is cooled down to 55°C, you can either pour right away, or add the antibiotic of your choice:

Antibiotic	Stock	Dilution	Final Concentration	Vol Added to 500 ml of Media
Ampicillin	100 mg/ml	1:1000	100 µg/ml	0.5 ml
Kanamycin	50 mg/ml	1:1000	50 µg/ml	0.5 ml
Chloramphenicol	50 mg/ml	1:3333	15 µg/ml	0.15 ml

- a. Make sure to mix well after adding the antibiotic and then pour.

Sodium Acetate (NaOAc), 3M, pH 5.2

1. Mix the following:

Reagent	Amount Needed
Sodium Acetate Trihydrate	204 g
dH ₂ O	400 ml

a. Make sure you are using sodium acetate trihydrate

2. pH the solution with glacial acetic acid to pH 5.2
3. Adjust final volume to 500 ml.
4. Store in glass bottles in 100 ml aliquots.

MgSO₄, 1M Solution

1. To make 500 ml, completely dissolve 123.24 g of MgSO₄ heptahydrate (FW = 246.48) in 450 ml distilled, MilliQ water.

a. Make sure you are using MgSO₄ heptahydrate.

2. Transfer to a graduated cylinder and bring up to final volume.
3. Filter sterilize into a 500 ml Filter Bottle using the disposable vacuum filtration systems (Thermo-Fisher Nalgene - 0.22 μ M, Rapid Flow Filter).

CaCl₂, 1M Solution

1. To make 500 ml, completely dissolve 55.49 g of CaCl₂ anhydrous (FW = 110.98) in 450 ml distilled, MilliQ water.

a. Make sure you are using CaCl₂

2. Transfer to a graduated cylinder and bring up to final volume.
3. Filter sterilize into a 500 ml Filter Bottle using the disposable vacuum filtration systems (Thermo-Fisher Nalgene - 0.22 μ M, Rapid Flow Filter).

MgCl₂, 1M Solution

1. To make 500 ml, completely dissolve 101.65 g of MgCl₂ hexahydrate (FW = 203.3) in 450 ml distilled, MilliQ water.

a. Make sure you are using MgCl₂ hexahydrate

2. Transfer to a graduated cylinder and bring up to final volume.
3. Filter sterilize into a 500 ml Filter Bottle using the disposable vacuum filtration systems (Thermo-Fisher Nalgene - 0.22 μ M, Rapid Flow Filter).

KCl, 1M Solution

1. To make 500 ml, completely dissolve 37.28 g of KCl (FW = 74.55) in 450 ml distilled, MilliQ water.
2. Transfer to a graduated cylinder and bring up to final volume.
3. Filter sterilize into a 500 ml Filter Bottle using the disposable vacuum filtration systems (Thermo-Fisher Nalgene - 0.22 μ M, Rapid Flow Filter).

NaCl, 5M Solution

1. To make 1 L, completely dissolve 292.2 g of NaCl (FW = 58.44) in 900 ml distilled, MilliQ water.
 - a. This will take a long time and you will need to bring the volume up to almost a full liter before the NaCl will dissolve.
2. Transfer to a graduated cylinder and bring up to final volume.
3. This is stored without sterilizing.

Orange G 6X Gel Loading Dye

1. Make a 6X stock (0.9%) by adding the Orange G dye to the glycerol in a 15 ml conical tube and shaking to mix completely.

Reagent	Amount Needed
Orange G dye	45 mg
30% Glycerol (filter sterilized)	10.0 ml

2. Aliquot 1 ml each into pre-labeled 1.7 ml tubes and store at room temperature or at -20C.

TE buffer

1. Mix the following:

Reagent	Amount Needed to make 500 ml	Amount needed to make 200 ml
1M Tris (pH 8)	5 ml	2ml
0.5M EDTA (pH 8)	1 ml	0.4 ml
dH ₂ O	494 ml	197.6 ml

2. Aliquot 10 ml each into 15 ml conical tubes.

Tris, 1M, pH 8

Reagent	Amount needed
Tris Base	121.14 g
HCl	~ 80 - 85 ml
dH ₂ O	up to 1L

1. Dissolve Tris Base in 800 ml dH₂O, while monitoring pH.
2. Slowly add HCl until the solution is pH 8
3. Bring final volume to 1L
4. Aliquot 200 ml into 250 ml bottles
5. Autoclave on liquid cycle, 30 minutes

2X Lysis Buffer

Reagent	Amount Needed	Final Concentration
KCl, 1M	100 mL	100 mM
Tris pH 8.2, 1M	20 mL	20 mM
MgCl ₂ , 1M	5 mL	5 mM
IGEPAL	9 mL	0.9%
Tween20	9 mL	0.9%
Gelatin	200 mg	0.02%
dH ₂ O	up to 1 L	-

Just before use, add 20 µl of 20 mg/mL Proteinase K((in water, store in frozen aliquots) per 1 mL 2X lysis buffer (or 2 µl of pK plus 198 µl 2X lysis buffer).

Soft Agar Freezing Buffer

1. Place a 1 L **glass** beaker on a scale and add:

Reagent	Amount needed
glycerol	300 g
agar	4 g
M9	up to 950 ml

- a. **You must use a glass beaker to heat the solution enough to dissolve the agar.**
2. Add stir bar and heat/stir to dissolve agar.
 - a. This may take a long time; need to heat up to 200°C-215°C.
 3. Allow solution to cool (still warm, but cool enough to handle).
 4. Transfer to graduated cylinder and bring up 1 L.
 5. Return solution to glass beaker and stir to mix.
 6. Transfer back to graduated cylinder and pour into to glass bottles, 100 ml per bottle. Autoclave 20 min.
 7. Store at room temperature

Cholesterol, for NGMA

unfiltered but sterile 5 mg/mL cholesterol in ethanol

1. Take an autoclaved sterile 250 mL bottle
2. Add 1 g of powdered cholesterol to the bottle
3. Add 200 mL of 100% ethanol to the bottle
4. Cap and shake to resuspend
5. Store at room temperature on the reagents shelf for up to six months

Cholesterol, for HTA

filtered sterile 5 mg/mL cholesterol in ethanol

1. Take a 50 mL conical tube.
2. Add 125 mg of powdered cholesterol to the tube.
3. Add 25 mL of 100% ethanol to the tube.
4. Cap and shake to resuspend.
5. Aliquot using a 25 mL syringe fitted with a filter (Millipore Millex-LG cat #SLLG025SS).
6. Dispense 1 mL of 5 mg/mL cholesterol through the filter into autoclaved sterile 1.7 mL microfuge tubes.
7. Cap and store at room temperature for up to six months
8. 125 μ L of 5 mg/mL cholesterol in ethanol should be added to 500 mL of K medium.

Diluting DNA Ladders, Invitrogen 1 kb Plus

To make 2.5 ml of 0.1 μ g/ μ l working stock from 1 tube of Invitrogen 1 kb Plus DNA Ladder (250 μ l, 1 μ g/ μ l):

1. In a 15 ml conical, combine:
 - a. 250 μ l Invitrogen 1 kb Plus DNA Ladder
 - b. 417 μ l 6X loading dye
 - c. 1,833 μ l dH₂O
2. Cap tube and vortex to mix.
3. Aliquot 250 μ l into 10 1.7 ml microfuge tubes.
4. Store one tube at room temperature and put the remaining aliquots in the DNA ladder box in the -20°C freezer.

Diluting DNA Ladders, NEB 1 kb

To make 10 ml of 0.05 μ g/ μ l working stock from 1 tube of NEB 1 Kb DNA Ladder (1000 μ l, 0.5 μ g/ μ l)

1. In a 15 ml conical, combine:
 - a. 1000 μ l NEB 1 Kb DNA Ladder
 - b. 1,667 μ l 6X loading dye
 - c. 7,333 μ l dH₂O
2. Cap tube and vortex to mix.
3. Aliquot 1000 μ l into 10 1.7 ml microfuge tubes.
4. Store one tube at room temperature and put the remaining aliquots in the DNA ladder box in the -20°C freezer.

NGMA Plates

Pre-autoclave:

	1 L	2 L	3L	4L	5L	6L	7L	8L	9L	10L
Peptone	2.5 g	5 g	7.5 g	10 g	12.5 g	15 g	17.5 g	20 g	22.5 g	25 g
NaCl	3 g	6 g	9 g	12 g	15 g	18 g	21 g	24 g	27 g	30 g
Agarose	7 g	14 g	21 g	28 g	35 g	42 g	49 g	56 g	63 g	70 g
Agar	10 g	20 g	30 g	40 g	50 g	60 g	70 g	80 g	90 g	100 g
Sterile water	975 ml	1950 ml	2925 ml	3900 ml	4875 ml	5850 ml	6825 ml	7800 ml	8775 ml	9750 ml

Note: dry ingredients can be premeasured into bottles which can then be poured into the MediaClave. Use some of the required water to wash out the bottles into the MediaClave.

Post-autoclave:

	1 L	2 L	3L	4L	5L	6L	7L	8L	9L	10L
1) 1 M KH_2PO_4 (K Phosphate Buffer)	25 ml	50 ml	75 ml	100 ml	125 ml	150 ml	175 ml	200 ml	225 ml	250 ml
2) Cholesterol (5 mg/ml in EtOH)	1 ml	2 ml	3 ml	4 ml	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml
3) 1 M CaCl_2	1 ml	2 ml	3 ml	4 ml	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml
4) 1 M MgSO_4	1 ml	2 ml	3 ml	4 ml	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml