**Autoclave procedures**

To autoclave **liquids** and **waste:**

Make sure all bottles containing liquid are slightly unscrewed such that steam can escape. Set autoclave to ‘Liquid cycle” and change sterilization time to 30 min, exhaust time to 0 min. Make sure to reset the jacket steam (red button) after the run is completed (about 1 hour later)

**Liquid cycle**

**\*30 min sterilization**

**\*0 min exhaust (none)**

To autoclave **solids** and **glassware**:

Set autoclave to ‘solids’ or ‘gravity’ cycle. Sterilization time should be set to 20 min, and exhaust time to 20 min.

**Gravity/solid cycle**

**\*20 min sterilization**

**\*20 min exhaust**

**Abbreviations**

‘Liquid media’ vs ‘agar plates’ – Media or liquid media is the broth form of a growth medium. To make liquid media, follow the normal recipe but make sure to NOT include agar. Agar media (ex. ‘KB agar’) refers to when agar is added to a liquid media to make petri dishes of media. Typically, we add about 7.5g of agar to a 500mL media. But this is not always the case, so make sure to check the recipe.

KB media -- King’s B Media. Used to cultivate pseudomonads. Iron limiting media which results in fluorescent siderophore expression.

YPD media – Yeast, Peptone, and Dextrose media. A broad media used to cultivate yeasts and fungi. In our lab, antibiotics are added to select for *Yarrowia*-like yeast isolates.

**Antibiotics & Antifungals**

|  |  |  |  |
| --- | --- | --- | --- |
| **Abbreviation** | **Name** | **Stock concentration** | **Typical media concentration** |
| **Rif** | Rifampicin | 100 mg/mL | 50 µg/mL |
| **Tet** | Tetracycline | 50 mg/mL | 10 µg/mL |
| **Gent** | Gentamycin | 50 mg/mL | 25 µg/mL |
| **Kan** | Kanamycin | 50 mg/mL | 50 µg/mL |
| **Strep** | Streptomycin | 100 mg/mL |  |
| **Chlor** | Chloramphenicol | 50 mg/mL | 30 µg/mL |
| **Amp** | Ampicillin | 50 mg/mL |  |
| **Nal** | Nalidixic Acid | 30 mg/mL | 15 µg/mL |
| **NFT** | Nitrofuratoin | 50 mg/mL | 50 µg/mL |
| **Nyst** | Nystatin (antifungal) | 35 mg/mL | 35 µg/mL |

**Media Abbreviations**

|  |  |  |
| --- | --- | --- |
| **KB** | King’s B media | Used to grow pseudomonads. Iron limited. |
| **LB** | Liquid broth media | Broad media for environmental bacteria |
| **TSB** | Tryptic soy broth | Broad media |
| **TSA** | Tryptic soy agar | Broad media |
| **YPD** | Yeast, Peptone, and Dextrose media | Used to grow yeasts/fungi |
| **BHI** | Brain Heart Infusion media | Broad media, used to grow host associated bacteria |
| **863** |  | Special medium for serratia symbiotica |
| **Leeds** | Leeds Acinetobacter media | Agar media with a pH indicator. Used to identify Acinetobacter. |
| **M9 Minimal media** | M9 Minimal media | Media with minimal nutrients used to test growth on various carbon sources. A carbon source must be added for growth. |
| **PMM** | Pseudomonas Minimal media | A nutrient limited minimal media specific for pseudomonads |

**Rifampicin stocks (100 mg / mL)**

* Dissolve 1 g in 10 mL of DMSO in hood.
* Vortex (and heat gently if needed) to dissolve
* Filter through 0.2 µm and aliquot.
* Store in -20 freezer.
* Add 500 µl / L for final concentration of 50 µg / mL

**3M Sodium Hydroxide (NaOH) stocks**

* Make 200 mL
* For 3M:
  + - 1M = 40 g in 1 L \* 3 = 120 g / 5 = **24g**
* Dissolve slowly in DI water in fume hood.
* Store at room temp.

**Nystatin (anti-fungal) stocks**

* Dissolve 350 mg in 10 mL of 70% EtOH.
* Add 1 mL / L to cool medium
* => 35 µg / mL
* Store in -20 freezer

**1M Magnesium Chloride (MgCl2) stock**

* Molecular weight = 95.21
* Make 200 mL
* 0.2 \* 95.21 = **19.04 g**
* Dissolve in DI water in the fume hood.
* NB: it does warm up a bit.
* Autoclave on fluid run for 30 mins.
* Aliquot into 1 mL tubes for daily use.

**M9 Minimal Salts 10X stock (for M9 media)**

* DI H2O 500 mL
* Na2HPO4 · 7H2O 35 g
* KH2PO4 15 g
* NaCL 2.5 g
* NH4Cl 5 g

**10X DNA gel-loading dye, 10mL**

* Glycerol 3.9 mL
* 10% (w/v) SDS 500 µL
* 0.5 M EDTA 200 µL
* Bromophenol blue 0.025 g
  + (Borrowed from Peter’s lab)
* Xylene cyanol 0.025 g
  + (Can be skipped)
* Bring to 10 mL total volume with DI H20
* Filter sterilize with syringe and 15mL falcon tube

**0.5M EDTA, 1L**

* MilliQ H2O 800 mL
* EDTA disodium salt 186.1 g
* NaOH tablets up to 2 g, added SLOWLY.

Add the EDTA to approximately 800mL of Milli-Q water and stir vigorously on magnetic stirrer. Adjust volume to 1L with Milli-Q water. Slowly add NaOH tablets (a few at a time) to adjust the pH to 8.0. The EDTA will not dissolve until the pH reaches 8.0.

**1x Phosphate buffered saline (PBS), 1L**

* DI H2O
* NaCl 8 g
* KCL 0.2 g
* Na2HPO4 1.44 g
* K2HPO4 0.24 g

\*pH to 7.4 using HCL

Add the ingrdients to approximately 900 mL of distilled water and stir vigorously on a magnetic stirrer to dissolve. Adjust volume to 1L using distilled water. Adjust pH to 7.4 using HCL. Autoclave for 20min.

**Tris-acetate-EDTA (TAE) Buffer (50X), 1L**

* Tris base 242.0 g
* Glacial acetic acid (Open in hood!) 57.1 g
* EDTA disodium salt 18.6 g
* DI H2O

Add the tris, EDTA, and glacial acetic acid to approximately 700mL of distilled water and stir until the contents are dissolved. Adjust the volume t0 1L using distilled water.

\*To make 1L of 1X TAE, add 20 mL 50X TAE to 980 mL distilled H2O

**Tris-borate-EDTA (TBE) Buffer (10X), 1L**

* Tris base 108 g
* Boric acid 55 g
* EDTA disodium salt 7.5 g
* DI H2O

Add the tris, EDTA, and boric acid to approximately 800mL of distilled water and stir until contents are dissolved. Adjust volume to 1L using distilleds water.

\*To make 1L of 1X TBE, add 100mL of 10X TAE to 900mL DI H2O

**King’s B (KB) agar or media (500 ml)**

* Distilled H2O 500mL
* Peptone 10 g
* K2HPO4 0.75 g
* MgSO4•7H2O 0.75 g
* Glycerol 5.0 ml
* Agar (if needed) 7.5 g

Measure 500 ml DI water into a 1 L glass media bottle. Add ingredients in order above (use syringe for glycerol) and swirl to mix. Autoclave on liquid run for 30 mins. Place in waterbath at 56ºC until cool enough to pour.

If adding rifampicin or other antibiotics/antifungals, wait until bottle is temperature of a warm cup of tea before adding. **For a final concentration of 25µg/mL rifampicin in 500mL of media, add 250µL of the 100mg/mL rif stock and swirl to stir**.

Optional additions:

* Rifampicin (25ug/ml) add 250µL stock to 500mL media.
* Nystatin (35 µg/mL) add 500µL stock to 500mL media.

**Liquid Broth (LB) media (500mL)**

* DI H2O 500 mL
* Tryptone 5.0 g
* Yeast Extract 2.5 g
* NaCl 5.0 g
* Agar (if needed) 6.5 g

Adjust pH to 7.4

\*autoclave on liquid cycle for 20min

**Yeast extract, Peptone, & Dextrose (YPD) Media (500mL)**

For the cultivation of Yarrowia and other fungi.

* DI H2O 500 mL
* Peptone 10 g
* Yeast extract 5 g
* Dextrose 10 g
* Agar 7.5 g

Antibiotics (for selection of Yarrowia isolates)

**To get: Add:**

15ug/mL tetracycline 250uL of stock

15ug/mL nalidixic acid 150uL of stock

25ug/mL chloramphenicol 250uL of stock

**Nitrogen Limited Leeds Agar**

**(Katie’s frankenmedia for Yarrowia)**

**(500mL)**

|  |  |
| --- | --- |
| * DI H2O | 500 mL |
| * Casein acid hydrolysate | 7.5 g |
| * Peptone | 2.5 g |
| * Sodium Chloride | 2.5 g |
| * L-phenylalanine | 0.50 g |
| * Ferric ammonium citrate | 0.20 g |
| * Phenol red | 0.01 g |
| * Glycerol | 6.65 mL |
| * Agar | 6.0 g |
| pH to 7.0 +/- 0.2 |  |

**Leeds Acinetobacter Agar Base (500mL)**

|  |  |
| --- | --- |
| * DI H2O | 500 mL |
| * Casein acid hydrolysate | 7.5 g |
| * Soya peptone | 2.5 g |
| * Sodium Chloride | 2.5 g |
| * Fructose | 2.5 g |
| * Sucrose | 2.5 g |
| * Mannitol | 2.5 g |
| * L-phenylalanine | 0.50 g |
| * Ferric ammonium citrate | 0.20 g |
| * Phenol red | 0.01 g |
| * Agar | 6.0 g |
| * pH to 7.0 +/- 0.2 |  |
|  |  |

**M9 Minimal Media (500mL)**

* DI H2O 450 mL
* M9 Salts (1x) 50 mL
* Agar 15 g

**Autoclave, then add:**

* 1M MgSO4 1.0 mL
* 1M CaCl2 0.1 mL (100uL)
* Carbon source

Possible carbon sources:

10% glucose 5mL

20% sucrose 10mL

20% fructose 10mL

**Pseudomonas Minimal Media (PMM) (500mL)**

* DI H2O 492.5 mL
* Glycerol 7.5 mL (leave out if testing other carbon source)
* L-Glutamine 2.5 g
* K2HPO4 0.75 g
* MgSO4 0.1 g
* Agar 7.5 g

**863 Media (500mL), liquid**

For the cultivation of *serratia symbiotica*

* DI H2O 500mL
* Glucose 5g
* Yeast Extract 5g
* Casein peptone (or tryptone) 5g

**868 Media (500mL), agar**

(same as 863 media, but with 1.7% agar)

* DI H2O 500mL
* Glucose 5g
* Yeast Extract 5g
* Casein peptone (or tryptone) 5g
* Agar 8.5 g

**Nitrofurantoin stocks (50 mg / mL)**

1. Make solution of:
   * 1500 mg Nitrofurantoin
   * 30 mL ddH2O
2. Aliquot into 2 mL tubes.
3. Store at 4°C.

**Aphid diet ingredients**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **NAME** | **Douglas (g/L)** | Date: |  |  |  |  |  |  |  |  |  |
| Alanine | 0.4455 |  |  |  |  |  |  |  |  |  |  |
| Arginine | 2.178 |  |  |  |  |  |  |  |  |  |  |
| Asparagine, H2O | 1.652 |  |  |  |  |  |  |  |  |  |  |
| Aspartic Acid | 1.664 |  |  |  |  |  |  |  |  |  |  |
| Cysteine | 0.3029 |  |  |  |  |  |  |  |  |  |  |
| Glutamic Acid | 1.103 |  |  |  |  |  |  |  |  |  |  |
| Glutamine | 2.192 |  |  |  |  |  |  |  |  |  |  |
| Glycine | 0.0751 |  |  |  |  |  |  |  |  |  |  |
| Histidine, HCl, H2O | 1.5722 |  |  |  |  |  |  |  |  |  |  |
| Isoleucine | 0.9839 |  |  |  |  |  |  |  |  |  |  |
| Leucine | 0.9839 |  |  |  |  |  |  |  |  |  |  |
| Lysine mono HCl (182.65g/mol) | 1.3699 |  |  |  |  |  |  |  |  |  |  |
| Methionine | 0.373 | fridge |  |  |  |  |  |  |  |  |  |
| Phenylalanine | 0.413 |  |  |  |  |  |  |  |  |  |  |
| Proline | 0.5757 |  |  |  |  |  |  |  |  |  |  |
| Serine | 0.5255 |  |  |  |  |  |  |  |  |  |  |
| Threonine | 0.8934 |  |  |  |  |  |  |  |  |  |  |
| Tryptophan | 0.5106 |  |  |  |  |  |  |  |  |  |  |
| Tyrosine | 0.0906 |  |  |  |  |  |  |  |  |  |  |
| Valine | 0.8786 |  |  |  |  |  |  |  |  |  |  |
| Sucrose | 171.15 |  |  |  |  |  |  |  |  |  |  |
| p-aminobenzoic acid | 0.1 |  |  |  |  |  |  |  |  |  |  |
| L-ascorbic acid | 1 |  |  |  |  |  |  |  |  |  |  |
| Biotin | 0.001 | fridge |  |  |  |  |  |  |  |  |  |
| D-calcium pantothenate | 0.05 |  |  |  |  |  |  |  |  |  |  |
| Choline chloride | 0.5 |  |  |  |  |  |  |  |  |  |  |
| Folic acid | 0.01 |  |  |  |  |  |  |  |  |  |  |
| i-Inositol | 0.42 |  |  |  |  |  |  |  |  |  |  |
| Nicotinamide (amide of niacin) | 0.1 |  |  |  |  |  |  |  |  |  |  |
| Pyridoxin HCl | 0.025 |  |  |  |  |  |  |  |  |  |  |
| Thiamine di-HCl | 0.025 |  |  |  |  |  |  |  |  |  |  |
| CuSO4 5H2O (0.1M) | 0.00254 | 102 μl |  |  |  |  |  |  |  |  |  |
| FeCl3 6 H2O (0.1M) | 0.01336 | 494 μl |  |  |  |  |  |  |  |  |  |
| MnCl2 4H2O (0.1M) | 0.00504 | 255 μl |  |  |  |  |  |  |  |  |  |
| NaCl | 0.01271 |  |  |  |  |  |  |  |  |  |  |
| ZnCl2 (0.1M) | 0.00417 | 306 μl |  |  |  |  |  |  |  |  |  |
| Calcium citrate | 0.1 |  |  |  |  |  |  |  |  |  |  |
| Cholesteryl benzoate | 0.025 |  |  |  |  |  |  |  |  |  |  |
| MgSO4, 7H2O | 2.42 |  |  |  |  |  |  |  |  |  |  |
| KH2PO4 | 2.5 |  |  |  |  |  |  |  |  |  |  |
| **THEN ADJUST pH TO 7.5 WITH ~14 ML NaOH 3M** |  |  |  |  |  |  |  |  |  |  |  |