Protocol: Media Preparation and Sterilization

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This protocol was made by last year's iGEM team, with some modifications made if necessary.

General Guidelines for labeling Agar Plates and Tubes

- Always label before adding any samples or media.
- Write clearly using block letters.
- Use permanent, waterproof markers
- Record full sample details in the lab notebook

Labeling Agar Plates

- Label on the bottom side (where the agar will be)
- Write along the edge of the plate
- Label should include:
    - Sample name or strain ID
    - Media type (e.g., LB, M9) and any additives (e.g., Ampicillin)
    - Date (YYYY-MM-DD)

Labeling Tubes

- Label the **body/side** of the tube, not just the cap.
- Include the following on the label:
    - Sample name or ID
    - Date (YYYY-MM-DD)

LB Media (1 L)

Ingredients:

- 10 g tryptone
- 5 g yeast extract
- 10 g NaCl
- 1 L distilled H_2O (not MQ water)

Instructions:

1. Place the vessel on a magnetic stirrer and dissolve all ingredients in 800 mL distilled H_2O.
2. Adjust the volume to 1 L with distilled H_2O using a graduated cylinder.
3. Autoclave following the instructions called Autoclave Procedure.

LB Agar (1 L)

Ingredients:

- 10 g tryptone
- 5 g yeast extract
- 10 g NaCl
- 15 g agar for bacteriology
- 1 L distilled H_2O (not MQ water)

Instructions:

1. Weigh the salts / powders, then put them in the Schott flasks.
2. Measure the water in a measuring cylinder, then add it to the Schott flask.

   ⚠️ **Do not add water before salts, as this will make them stick to the side**.

3. Autoclave following the instructions called Autoclave Procedure.
4. Cool to ~40°C before adding antibiotics, or until cool enough you can barely hold it with your hands.

   ⚠️ **Do not add antibiotics above 50°C** to prevent degradation.

5. Pour media with/without antibiotics following the instructions called Pouring Agar Plates.

Tips from Sigma Aldrich:

- If not mixed properly the powder can bake on the bottom of the bottle. Undo the lid about half a turn; add some autoclave tape and then autoclave
- After autoclaving ensure that the lid is done up tight and do not allow it to cool below 45-50 degrees. Agar generally sets at about 40 degrees. As a guide if you can hold the bottle comfortably in your hand then it is ready to pour and may actually be too cool already
- Don't add the antibiotic when the agar is too hot, this can affect the antibiotic stability and half life, particularly Ampicillin. Add the correct amount of antibiotic and mix by gentle swirling. Avoid getting bubbles as this will result in bubbly or uneven plates.

- Many lab bottles have a blue or clear plastic rim which can go missing or get burnt by flaming. These blue rims prevent the agar running down the bottle when pouring the plates and keep an air tight seal after autoclaving. Make sure the rim is intact.

Antibiotic Stock Solutions

Ampicillin (100 mg/mL)

- Label all tubes clearly!
- In a 50 mL Falcon tube, dissolve 2 g ampicillin salt in 20 mL MilliQ H_2O.
- Vortex carefully to dissolve the ampicillin.
- Filter sterilize using a syringe and a 0.2 µm filter.
- Transfer the solution directly to a sterile 50 mL falcon tube.
- Store the solution at 4°C.

Pouring Agar Plates

- Label all plates clearly!

  ⚠️ **Do not discard the bag after taking out the plates as we will use them to store plates after pouring agar.**

- Melt LB agar in a microwave. Take out and stir every minute to avoid pressure build up and explosions.

- Cool agar to ~40°C before adding antibiotics. Add antibiotic stock (1:1000 dilution; e.g., 100 µL of stock per 100 mL agar → final concentration will be 100 µg/mL).

  ⚠️**Cool it enough to make sure that you are able to hold in your hands.**

- Swirl gently to mix (do not create bubbles).

  ⚠️ Take care when microwaving agar to avoid **boiling over**. Heat in short bursts and vent containers.

- Pour ~20 mL into each sterile Petri dish (**fill them up to halfway - Better thicker than thinner to prevent the plates from drying out**).
- Allow to solidify, then store upside down at 4°C in sealed bags or containers (preferably the bags they were in before they were opened).

Inducers

IPTG stock solution (100 mM)

- Label all tubes clearly!
- Weigh 0.238 g of IPTG.
- In a 15 mL tube, dissolve 0.238 g of IPTG in 10 mL MilliQ H_2O.
- Vortex if necessary.
- Pass the fully dissolved solution with a syringe through a 0.22 µm filter and into a fresh, sterile container.
- Aliquot in 2 mL tubes (fill up to max 1.5 mL as liquids expand when freezing). → avoid repeated thawing and freezing.
- Store at 4°C for short term and -20°C for longer term, protected from light!

Use 1:100 dilution for induction.

Lower temperature if expressing protein!

$CaCl_2$ stock solution (50 mM)

Option 1: *$CaCl_2$ (Mw: 110.98 g/mol)*

Final Volume (mL)	$CaCl_2$ needed (g)	$CaCl_2 \cdot 2H_2O$ needed (g)
100	0.55	0.74
200	1.10	1.47
500	2.77	3.68

Recipe (per volume):

1. **Weigh out $CaCl_2$** as per the table above.
2. **Dissolve** in ~80% of the final volume of distilled water.
3. **Mix until fully dissolved.**
4. **Adjust volume** to final mark (100, 200, or 500 mL).
5. **Sterilize** if needed (filter or autoclave). <u>For smaller volumes filter!!</u>

LB Media Recipes (various volumes)

Final Volume	Tryptone	Yeast Extract	NaCl	Distilled H_2O (not MQ water)
100 mL	1 g	0.5 g	1 g	100 mL
200 mL	2 g	1 g	2 g	200 mL
250 mL	2.5 g	1.25 g	2.5 g	250 mL
500 mL	5 g	2.5 g	5 g	500 mL
1 L	10 g	5 g	10 g	1 L
1.5 L	15 g	7.5 g	15 g	1.5 L
2 L	20 g	10 g	20 g	2 L

LB Agar Recipes (various volumes)

Final Volume	Tryptone	Yeast Extract	NaCl	Agar	Distilled H_2O (not MQ water)
100 mL	1 g	0.5 g	1 g	1.5 g	100 mL
250 mL	2.5 g	1.25 g	2.5 g	3.75 g	250 mL
500 mL	5 g	2.5 g	5 g	7.5 g	500 mL
1 L	10 g	5 g	10 g	15 g	1 L
1.5 L	15 g	7.5 g	15 g	22.5 g	1.5 L
2 L	20 g	10 g	20 g	30 g	2 L