PREPARATION

Preparing Working Media

Objective

To "finish" several common reagents by adding final ingredients.

Background

Because not all components of reagents are heat-stable, some must be added after autoclaving but before use of the reagent. Other reagents are kept in stock as a concentrated form (e.g. MBTA) and must be diluted. This protocol describes how to make ready-to-use versions of **Phage Buffer**, **7H9** and **Top Agar**.

Approximate Time Needed

~**5 minutes** for each solution

Materials Needed

For **Working Phage Buffer** (Phage Buffer w/ 1 mM $CaCl_2$):
- 100 ml Phage Buffer (see **Preparing Phage Buffer** protocol)
- 1 ml 0.1 M $CaCl_2$

For **"Dilution" 7H9** (7H9 w/ 2 mM $CaCl_2$, for use in making Top Agar only):
- 100 ml 7H9 (see **Preparing 7H9** protocol)
- 2 ml 0.1 M $CaCl_2$

For **Top Agar**:
- 50 ml MBTA (see **Preparing MBTA** protocol)
- 50 ml "Dilution" 7H9 (see above)

Sterile pipettes

Microwave

Helpful Tips

- *Working* Phage Buffer should be used for all experiments and dilutions. Make sure you have added $CaCl_2$ to your Phage Buffer before using it.
- "Dilution" 7H9 has twice the normal concentration of $CaCl_2$ (2 mM) because it will be used to dilute MBTA, which has no 7H9. Thus, once "Dilution" 7H9 is mixed with MBTA to make Top Agar, the final concentration of $CaCl_2$ in the Top Agar will be the normal 1 mM.
- Top Agar is best when made just before plating. If, however, you have leftover Top Agar from a previous experiment and wish to use it, microwave until completely melted, then allow it to cool to about 55°C before using. Working with agar that is too hot will kill bacteria and phage, and working with it too cool will make chunky plates that are difficult to analyze.

Procedure: Working Phage Buffer

1. Sterilely add the 1 ml of 0.1 M $CaCl_2$ to your 100 ml bottle of Phage Buffer. The final concentration of $CaCl_2$ in the buffer should be 1 mM, and if you have smaller or larger bottles of Phage Buffer, the volume of $CaCl_2$ to be added can be adjusted accordingly.

2. Securely close the bottle and invert several times to mix.

3. Clearly mark the label of the bottle to indicate $CaCl_2$ has already been added (e.g., add "+ 1mM $CaCl_2$" to the Phage Buffer label).

4. Use as is in experiments/dilutions.

Procedure: "Dilution" 7H9

1. Sterilely add **2** ml of 0.1 M $CaCl_2$ to your 100 ml bottle of 7H9. The final concentration of $CaCl_2$ in the buffer should be **2** mM (see second Helpful Tip above), and if you have smaller or larger bottles of 7H9, the volume of $CaCl_2$ to be added can be adjusted accordingly.

2. Securely close the bottle and invert several times to mix.

3. Clearly mark the label of the bottle to indicate $CaCl_2$ has already been added (e.g., add "+ 2mM $CaCl_2$" to the 7H9 label).

4. Use as is for making Top Agar (see below).

Procedure: Top Agar

1. Each normal-sized petri dish that will be plated requires about 5 ml of Top Agar. Figure out how much Top Agar you need, divide by two, and that is the amount of MBTA to start with.

2. Heat an appropriate amount of MBTA in a microwave until completely liquefied. This will take 1-2 minutes for 50 ml, and you should pause the microwave and shake the bottle vigorously to mix every 30 seconds or so. (Note: make sure the cap on the bottle is not airtight when microwaving, or pressure can build up with bad consequences.)

3. Remove the melted MBTA from the microwave.

4. Sterilely add an equal volume of "Dilution" 7H9 to your MBTA.

5. Cap and shake the bottle to mix. The room temperature 7H9 mixed with the hot MBTA will yield a solution of about 55-60°C.

6. The Top Agar is now ready to mix with cells and pour on a plate. Use immediately, or it will cool further and begin to solidify before plates are poured. (If this happens, just re-microwave until it's liquid again, wait until it's about 55°C, then continue.)

7. If you have a significant leftover amount of top agar, see the third Helpful Tip above.