**Phage Buffer (not working phage buffer)**

Preparation Time ~ one hour

Materials

Autoclave and autoclave tape

Glass bottles with caps suitable for autoclaving (Wheaton 500 ml work well)

Large beaker (1500-2000 ml) or divide the following ingredients as needed to suit smaller beakers

Stir plate and stir bar

10 ml 1M Tris, pH 7.5

10 ml 1M MgSO4

4 g NaCl

980 ml ddH2O

Procedure

Combine all ingredients into a beaker.

Stir for approximately five minutes or until combined thoroughly.

Aliquot into glass bottles.

**\*Note-** for making working phage buffer, aliquot 99 ml of phage buffer into bottles.

Autoclave for appropriate amount of time.

**\*Note-** if using a pressure cooker for autoclaving, run the unit for 20 minutes after reaching full pressure.

**Working Phage Buffer (ready to use)**

Materials

Sterile pipette and pipette pump

Sterile 0.1 M CaCl2

Sterile 99 ml phage buffer

Procedure

1. Sterilely add 1 ml of 0.1 M CaCl2 to the 99 ml bottle of phage buffer.
2. Cap the bottle and invert it several times to mix the solution thoroughly.
3. Label the bottle to indicate +1mM CaCl2.

**Carbenicillin (CB)**

50 ml

Materials

1000x CB

DI water

Filter sterilizer

Sterile tubes with caps

Balance with weigh boats

Dry media scoop

100 ml graduated cylinder

125 ml flask

Stirrer/stir bar

Procedure

Measure and pour 50 ml of DI water into the flask.

Measure 2.5 g of CB media and add to the flask.

Stir thoroughly using the stirrer and stir bar.

Filter sterilize and aseptically dispense into sterile tubes.

**Cycloheximide (CHX)**

50 ml

Materials

Cycloheximide stock dry media

DI water

Filter sterilizer

Sterile tubes with caps

Balance with weigh boats

Dry media scoop

100 ml graduated cylinder

125 ml flask

Stirrer/stir bar

Procedure

Measure and pour 50 ml of DI water into the flask.

Measure .5 g of CHX media and add to the flask.

Stir thoroughly using the stirrer and stir bar.

Filter sterilize and aseptically dispense into sterile tubes.

**Tween 20%**

50 ml

Materials

Tween80

DI water

Filter sterilizer

Sterile 125 ml flask with foil cap

10 ml graduated cylinder

50 ml graduated cylinder

125 ml working flask

Stirrer/stir bar

Procedure

Use the 50 ml graduated cylinder to add 30 ml DI water and the stir bar to the working flask.

Measure 10 ml of Tween80 into the graduated cylinder.

Place the working flask on the stirring plate (no heat) and slowly add the Tween80 while stirring.

Once the mixture has thoroughly mixed, pour into the 50 ml graduated cylinder. Add DI water to a final volume of 50 ml.

Filter sterilize and aseptically transfer to the sterile 125 ml flask and cover. Label Tween20.

**.1M CaCl2**

Materials

Autoclave and autoclave tape

Glass bottles with caps suitable for autoclaving (Wheaton 500 ml work well)

ddH2O

100 ml graduated cylinder

Large Beaker

Stir plate and stir bar

1.47 g CaCl2-2H2O (dihydrate) **-or-** 1.11 g CaCl2 anhydrous

Procedure for making 100 ml final solution

To 90 ml of ddH2O in a beaker add the measured amount of CaCl2.

Dissolve on stir plate.

Pour the dissolved solution into a 100 ml graduated cylinder and fill with ddH2O to 100 ml.

Add the solution to a bottle with the cap loosened and add autoclave tape.

Label as .1 M CaCl2 sterile.

Autoclave.

**7H9 Media with 2mM CaCl2**

Materials

10 glass bottles suitable for autoclave.

4.7 g Middlebrook 7H9 broth dry media

5 ml 40% glycerol

2 ml of 0.1 M CaCl2

900 ml DI water

Two liter flask/beaker

Glass stir rod

1000 ml graduated cylinder

Autoclave and autoclave tape

Hot plate/stirrer/stir bar

Procedure for making ~ 1L

Fill the flask/beaker with DI water.

Add the CaCl2.

Use the glass stir rod to dispense glycerol into the flask/beaker.

Measure and add the 7H9 dry media to the flask.

Use the stir bar on the stirring plate to mix approximately 10 minutes.

Aliquot into glass bottles and autoclave.

**MiddleBrook Top Agar (MBTA) (not working media)**

Materials

4.7 g 7H9 broth dry media

7.0 g BactoAgar

DI water

1000 ml graduated cylinder

2000 ml flask or beaker

Hot plate/stirrer/stir bar

18 glass bottles suitable for autoclave

Autoclave and autoclave tape

Procedure

Add 7H9 dry media and BactoAgar to the flask/beaker.

Fill to 900 ml with DI water.

Place stir bar in the container on a stirring hot plate and mix thoroughly.

Heat to boiling to evenly disperse media.

Aliquot 50 ml into glass bottles suitable for autoclave.

**Top Agar (working media)**

Materials

50 ml of MBTA

50 ml of 7H9 with 2 mM CaCl2

Sterile 250 ml flask with sterile stir bar

Microwave oven

Stirring Plate

Sterile Pipettes/ Pipette Pump

Procedure

Melt 50 ml of MBTA in the microwave oven and aseptically dispense into the sterile flask containing the sterile stir bar.

Add 50 ml of 7H9 w/ 2 mM CaCl2 to the flask.

Stir thoroughly and allow to cool to 550 C before using.

**LB++ Agar (Bottom agar for phage discovery lab)**

Approximately One LIter of LB agar plus carbenicillin (CB) plus cycloheximide (CHX)

Materials

40.0 g LB Agar dry media

1 ml sterile carbenicillin (CB)

1 ml sterile cycloheximide (CHX)

960 ml DI water

1 L graduated cylinder

Two Liter Flask

Aluminum Foil

Balance and weigh boats

Dry media scoop

Hot plate stirrer/stir bar

\*Water bath set to 550 C

Autoclave/autoclave tape

Sterile Petri plates (approximately 40 plates)

Procedure

Use the graduated cylinder to measure and pour the DI water into the flask.

Measure out the LB agar dry media and add to the flask.

Cover the flask with foil and autoclave.

Place the sterilized flask in the water bath for approximately 10 minutes to cool.

Add 1 ml of sterile carbenicillin (CB) to the flask and swirl to mix thoroughly.

Add 1 ml of sterile cycloheximide (CHX) to the flask and swirl to mix thoroughly.

Aseptically pour plates with enough agar to cover the bottom of the plate.

Allow the plates to cool before turning over and storing upside down at 40 C. Store in plastic wrap or plastic sleeves for extended periods.