## Phage Buffer (<u>not working phage buffer)</u>

Preparation Time ~ one hour

<u>Materials</u>
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 $MgSO_4$
4 g NaCl
980 ml dd$H_2O$

<u>Procedure</u>
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 (<u>ready to use</u>)

<u>Materials</u>
Sterile pipette and pipette pump
Sterile 0.1 M $CaCl_2$
Sterile 99 mL phage buffer

<u>Procedure</u>
1. Sterilely add 1 mL of 0.1 M $CaCl_2$ 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 $CaCl_2$.

## 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 0.5 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. Label 20% Tween80.

## 0.1M $CaCl_2$

Materials

Autoclave and autoclave tape
Glass bottles with caps suitable for autoclaving (Wheaton 500 mL work well)
dd$H_2O$
100 mL graduated cylinder
Large Beaker
Stir plate and stir bar
1.47 g $CaCl_2$-$2H_2O$ (dihydrate) **-or-** 1.11 g $CaCl_2$ anhydrous

Procedure for making 100 mL final solution

To 90 ml of dd$H_2O$ in a beaker add the measured amount of $CaCl_2$.
Dissolve on stir plate.
Pour the dissolved solution into a 100 ml graduated cylinder and fill with dd$H_2O$ to 100 ml.
Add the solution to a bottle with the cap loosened and add autoclave tape.
Label as 0.1 M $CaCl_2$ sterile.
Autoclave.

## 7H9 Media with 2mM $CaCl_2$

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 $CaCl_2$
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.
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. After cooling, add 2 mL 0.1 M $CaCl_2$

## 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 $CaCl_2$
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 $CaCl_2$ to the flask.
Stir thoroughly and allow to cool to $55^0$ 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 with stir bar
Aluminum foil
Balance and weigh boats
Dry media scoop
Hot plate stirrer/stir bar
*Water bath set to $55^0$ 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. Stir and heat until clear. Watch out for boiling over.
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 $4^0$ C. Store in plastic wrap or plastic sleeves for extended periods.