8/3/2010

Media for culturing biofilms in minimal media

1 M $CaCl_2$ solution

- 7.35 g of $CaCl_2 \cdot 2\ H_2O$
- 50 mL DI water

Filter sterilize

1 M $MgSO_4$ solution

- 12.32 g of $MgSO_4 \cdot 7\ H_2O$
- 50 mL DI water

Filter sterilize

Bushnell-Haas Salts (w/out carbon source)

- 0.4 g of KH_2PO_4
- 0.4 g of K_2HPO_4
- 0.4 g of $(NH_4)_2\ SO_4$ or NH_4NO_3
- 0.002 g of $FeCl_3$
- 318 mL of DI H_2O

Autoclave at 15 psi and 121C for 30 minutes

After the media has cooled ***completely*** add:

- 665 uL of 1 M $MgSO_4$ solution
- 72 uL of 1 M $CaCl_2$ solution

Bushnell-Haas Media with glucose

- 39.75 mL of Bushnell-Haas Salts
- 250 uL of 40% glucose solution
- 10 mL of sterile DI water

Cyclohexanecarboxylic acid solution

Only work with cyclohexanecarboxylic acid in the hood! It is volatile and smells very bad. Move a scale that has sliding doors to block airflow into the hood for accurate measurements.

- 0.075 g of cyclohexanecarboxylic acid
- 50 mL of DI water

Filter sterilize

Bushnell-Haas Media with cyclohexanecarboxylic acid

- 39.75 mL of Bushnell-Haas Salts
- 4 mL of cyclohexanecarboxylic acid solution
- 6.25 mL of sterile DI water

Casamino Acid Solution

- 0.18 g Casamino acid
- 9 mL of DI water

Filter sterilize

Bushnell-Haas Media with glucose and casamino acids

- 39.75 mL of Bushnell-Haas Salts
- 250 uL of 40% glucose solution
- 3.75 mL of sterile DI water
- 6.25 mL of casamino acid solution

0.10 M NaOH solution

BE VERY CAREFUL WHEN MIXING! SOLID NaOH BURNS YOUR SKIN BUT YOU MAY NOT FEEL IT AT FIRST! MAKE SURE ANY SPILLS ARE CLEANED UP VERY WELL!

- 0.2 g of NaOH
- 50 mL of sterile water

Filter Sterilize

Bushnell-Haas Media with cyclohexanecarboxylic acid adjusted to pH of 9

- 39.75 mL of Bushnell-Haas Salts
- 4 mL of cyclohexanecarboxylic acid solution
- 6.25 mL of sterile DI water

Test how much 0.1 M NaOH must be added to 1 mL of media to raise it to a pH of 9 (add 1 uL of base at a time)

Scale up the amount of 0.1 M NaOH solution to add to the remaining volume of media (approaching final amount slowly)

Today approximately 20 uL of 0.1 M NaOH had to be added to 1 mL to get to a pH between 8 and 9 (tested with pH paper) and 675 uL of 0.1 M NaOH was added into the 45 mL of remaining media.