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Minimal Basal Medium (liquid) V.2

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A defined artificial seawater medium for growing bacteria.

MBM.docx

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Minimal basal medium

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Increase or decrease amount proportionally to prepare stock quantities as needed. Add dry reagents slowly to allow for dissolution in between each addition. Solutions will be prepared and autoclaved separately, except for carbon and vitamins which are filter sterilized through 0.2 micron PES membranes. Each autoclaved solution should be cooled completely before combining - remember to autoclave extra bottles or graduated cylinders as needed for measuring and mixing. The carbon source should only be added after autoclaved solutions have cooled; it is filter sterilized into the medium and can be added during mixing or at any time before setting up a culture. Extra solutions can be stored frozen or at 4 Celsius. This recipe is for preparing 1 L of liquid medium, but plates can be made as well with slight variation.

:

Acid wash all glassware with 10% HCl and rinse well with dH_2O.

Sea Salt Solution

1 Add 20 g Sigma Sea Salts into a 1 L glass bottle.

2 Add 699 mL dH_2O to sea salts in bottle and mix well to dissolve.

Add a stir bar to bottle if needed to mix and dissolve salts.

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Remember if adding Bacto Agar that this will not dissolve until solution is autoclaved.

3 Set prepared sea salt solution aside until ready to autoclave.

FeEDTA Stock

4 Add 50 mg FeEDTA into a 250 mL glass bottle.

5 Add 100 mL dH_2O to FeEDTA in bottle and mix well to dissolve (stock concentration is 1.36 mM).

6 Filter-sterilize FeEDTA solution using a 0.2 micron PES membrane filter.

Basal Medium

7 Add 150 mL of 1 M Tris HCl, pH 7.5, into a 1 L glass bottle.

Accurate pH is very important!

8 Add 0.34 g NH_4Cl into the same bottle and dissolve.

9 Add 182.98 mg K_2HPO_4 into the same bottle and dissolve.

10 Add 375 mL dH_2O to the same bottle and mix well.

11 Set prepared basal medium aside until ready to autoclave.

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Autoclave

12 Autoclave sea salt solution and basal medium.

Remember to throw in extra bottles and/or graduated cylinders if needed.

Don't forget to loosen the lids before and tighten the lids after!

Do not combine solutions before autoclaving.

13 Cool autoclaved solutions to room temperature.

Mix

14 Add 50 mL of FeEDTA stock to the bottle of sea salt solution and mix well.

15 Add 250 mL basal medium to the bottle of sea salt solution and mix well.

16 Add 1 mL of vitamin supplement the the bottle of sea salt solution and mix well.

MBM Vitamin Supplement
by Christa Smith,
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PREVIEW RUN

16.1 Measure 950 mL dH_2O into a 1 L glass bottle.

16.2 Dissolve 2 mg biotin into the bottle.

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16.3 Dissolve 2 mg folic acid into the bottle.

16.4 Dissolve 10 mg pyridoxine-HCl into the bottle.

16.5 Dissolve 5 mg riboflavin into the bottle.

16.6 Dissolve 5 mg thiamine into the bottle.

16.7 Dissolve 5 mg nicotinic acid into the bottle.

16.8 Dissolve 5 mg pantothenic acid into the bottle.

16.9 Dissolve 0.1 mg cyanocobalamin into the bottle.

6.10 Dissolve 5 mg *p*-aminobenzoic acid into the bottle.

6.11 Bring final volume to 1 L with dH_2O.

6.12 Filter sterilize final vitamin solution through a 0.2 micron PES membrane.

6.13 Store frozen in 1 mL aliquots.

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Finish

17 Store prepared medium at 4 Celsius.

Prepared medium can be filtered (0.2 micron PES membrane) if needed.

Carbon source

18 A carbon source can be added to the finished minimal medium.

Carbon source (type and concentration) can be varied as needed.

Carbon source stock solution should be filter-sterilized through a 0.2 micron PES membrane filter and stored frozen until needed.

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