Minimal Basal Medium (liquid)

Christa Smith

Abstract
A defined artificial seawater medium for growing bacteria.

Citation: Christa Smith Minimal Basal Medium (liquid). **protocols.io**
dx.doi.org/10.17504/protocols.io.jvdcn26
Published: 28 Jun 2018

Guidelines

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.

Before start

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

Protocol

Sea Salt Solution

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

Sea Salt Solution

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

NOTES

Christa Smith 15 Sep 2017
Add a stir bar to bottle if needed to mix and dissolve salts.

Christa Smith 15 Sep 2017
Remember if adding Bacto Agar that this will not dissolve until solution is autoclaved.

Sea Salt Solution

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

FeEDTA Stock

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

FeEDTA Stock

Step 5.
Add 100 mL dH_2O to FeEDTA in bottle and mix well to dissolve.

FeEDTA Stock

Step 6.
Set prepared FeEDTA stock aside until ready to autoclave.

Basal Medium

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

NOTES

Christa Smith 20 Sep 2017

Accurate pH is very important!

Basal Medium

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

Basal Medium

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

Basal Medium

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

Step 11.
Set prepared basal medium aside until ready to autoclave.

Autoclave

Step 12.
Autoclave each of the 3 prepared solutions (Sea salt solution, FeEDTA stock, and basal medium).

NOTES

Christa Smith 15 Sep 2017

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

Christa Smith 15 Sep 2017

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

Christa Smith 20 Sep 2017

Do not combine the 3 solutions before autoclaving.

Autoclave

Step 13.

Cool autoclaved solutions to room temperature.

Mix

Step 14.

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

Mix

Step 15.

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

Mix

Step 16.

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

PROTOCOL

. MBM Vitamin Supplement

CONTACT: Christa Smith

Step 16.1.

Measure 950 mL dH_2O into a 1 L glass bottle.

Step 16.2.

Dissolve 2 mg biotin into the bottle.

Step 16.3.

Dissolve 2 mg folic acid into the bottle.

Step 16.4.

Dissolve 10 mg pyridoxine-HCl into the bottle.

Step 16.5.
Dissolve 5 mg riboflavin into the bottle.

Step 16.6.
Dissolve 5 mg thiamine into the bottle.

Step 16.7.
Dissolve 5 mg nicotinic acid into the bottle.

Step 16.8.
Dissolve 5 mg pantothenic acid into the bottle.

Step 16.9.
Dissolve 0.1 mg cyanocobalamin into the bottle.

Step 16.10.
Dissolve 5 mg *p*-aminobenzoic acid into the bottle.

Step 16.11.
Bring final volume to 1 L with dH_2O.

Step 16.12.
Filter sterilize final vitamin solution through a 0.2 micron PES membrane.

Step 16.13.
Store frozen in 1 mL aliquots.

Finish

Step 17.
Store prepared medium at 4 Celsius.

NOTES

Christa Smith 15 Sep 2017

Prepared medium can be filtered (0.2 micron PES membrane) as needed to remove any precipitates that may form over time.

Carbon source

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

NOTES

Christa Smith 20 Sep 2017

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

Christa Smith 20 Sep 2017

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