Mar 20, 2020

Preparing 1/2 SŠ Algal Inorganic Nutrient Medium

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In Development dx.doi.org/10.17504/protocols.io.bdzai72e

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ABSTRACT

This protocol describes preparation of 1/2 SŠ inorganic nutrient medium for *C. vulgaris, D. quadricauda,* and other fresh water microalgae. The medium is prepared out of sterile components inside sterile laminar flow hood. The medium recidpe is based on [Zachleder & Šetlík, 1982].

EXTERNAL LINK

https://app.labstep.com/sharelink/ddc19dd9-4958-42fb-818d-9238c4384ec8

GUIDELINES

This protocol describes preparation of a culture medium derived from 1/2 SŠ inorganic nutrient medium [Zachleder & Šetlík, 1982] for growth of fresh water microalgae on a shaking orbital incubator. The solution of the 1/2 SŠ medium (0.5 g/l) is prepared by mixing stocks of solutions of individual components in autoclaved deionized water. The final solution pH is adjusted by KOH and filter sterilized.

The protocol described here differs slightly from the source recipe. The original medium recipe is aimed at producing up to 6 g/l (dry weight) culture density. We use the cells for imaging and there is no need to produce such dense cultures. Therefore the nutrients were diluted 12 times in this protocol and the medium can only support cultures up to 0.5 g/l (dry weight) culture density. The original recipe does not include source of carbon, assuming sufficient gas exchange and reliance on atmospheric CO_2. The flask-based culture conditions limit atmospheric gas exchange. This protocol therefore adds 0.83 mM $NaHCO_3$ as a source of carbon.

The whole process should take about 1 hour.

MATERIALS TEXT

Autoclaved deionized water in glass bottle (0.5 l)

1/2 SŠ Stock KNO_3 (1.83 M, filter sterilized)

1/2 SŠ Stock KH_2PO_4 (174 mM, filter sterilized)

1/2 SŠ Stock $MgSO_4 \cdot 7H_2O$ (82.8 mM, filter sterilized)

Sodium Bicarbonate (500 mM, filter sterilized)

1/2 SŠ Stock Fe·Na·EDTA (9.1 µM, filter sterilized)

1/2 SŠ Stock $CaCl_2$ (66 µM, filter sterilized)

1/2 SŠ Stock Trace Elements (83 µl/l, filter sterilized)

HI-98100 Checker Plus pH Tester or other pH meter. Make sure the pH meter is calibrated before its use.

Potasium Hydroxide (2M, filter sterilized) for pH titration.

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15 ml Falcon tube

Pipettes and tips

SAFETY WARNINGS

The constituents of the medium are chemicals, which must be handled and stored according to local regulations. Potassium hydroxide (KOH) is caustic and stands out in its health and safety risks. Handle KOH with care. Avoid spilling it. Protect your eyes and hands with suitable personal protective equipment. Wipe away any spillage.

BEFORE STARTING

Ensure that all components are prepared and filter-sterilized. Autoclave deionized water in a glass bottle.

This protocol adds sodium bicarbonate, which is not in the original protocol and which was added as source of carbon for the algae. Autoclaving destroys sodium bicarbonate. It causes a chemical reaction converting the bicarbonate to into carbonate and change in pH.

Preparatory Steps

1 Prepare the components listed int he following table. Make sure all constituents have been sterilized and that the deionized water has been autoclaved.

Work in a sterile laminar flow hood.

Component	Quantity	Unit	Note
Final Volume	503	ml	
Maximum Dry Culture Weight	0.5	g/l	
Deionized Water	500	ml	Autoclaved
1/2 SŠ Stock KNO_3 (1.83 M)	833	µl	
1/2 SŠ Stock KH_2PO_4 (184 mM)	417	µl	
1/2 SŠ Stock $MgSO_4 \cdot 7H_2O$ (82.8 mM)	417	µl	
Sodium Bicarbonate (500 mM)	833	µl	Do not autoclave
1/2 SŠ Stock Fe·Na·EDTA (9.1 µM)	417	µl	Do not autoclave
1/2 SŠ Stock $CaCl_2$ (66 µM)	417	µl	
1/2 SŠ Trace Elements (1 ml/l)	42	µl	
2M KOH	20	µl	The goal is to get pH 7.5

Constituents of the 1/2 SŠ inorganic nutrient medium.

2 Autoclave 500 ml of deionized water in a glass clean bottle.

Let it cool down.

3 Prepare all the reagents and labware.

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Medium Preparation

4 Work in a sterile laminar flow hood.

Add components listed in table in STEP 1 into the autoclaved deionized water in the order listed in the table. Do not add KOH yet.

5 Adjust the pH to 7.5 using KOH.

5.1 Take a small aliquot (1.5 ml) of the medium into a 15 ml Falcon tube.

5.2 Measure the pH of the medium inside the Falcon tube using the pH meter.

5.3 Empty the Falcon tube.

5.4 Add a small aliquot (5µl) of 2 M KOH to the medium. Mix well by closing the bottle and shaking or turning up side down a few times.

5.5 Repeat by going back to STEP 5.1, until pH reaches a value of approximatelly 7.5.

6 Label the bottle (1/2 SŠ, initials, date, pH).

7 Store at 4 °C in dark.

Warm up to room temperature each time before use.

Only open in a sterile cabinet.

Summary

8 This protocol describes the preparation of a medium for growth of freshwater algae on an orbital shaking incubator. The medium is to be used to dilute the growing cultures at regular intervals. The medium needs to be stored in a fridge at 4 °C and warmed up each time to room temperature to minimize the stress to the culture.

References

Protocol for preparing the medium using filter sterilization: Preparing 1/2 SŠ algal inorganic nutrient medium

1/2 SŠ inorganic nutrient medium: Recipe from the Culture Collection of Autotrophic Organisms

Zachleder & Šetlík, 1982: The original article introducing this medium

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