May 28, 2020

Standard Volvox Medium

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ABSTRACT

This is a protocol for the execution of a Standard Volvox Medium recipe. Starr's (1969) modification of Provasoli & Pintner's Medium (1959), as further modified by Starr, Kochert and Kirk at various times.

GUIDELINES

Description

Class of Chemicals	Stock Solution	Stock Concentration	Final Concentration
Macro minerals, N-source, and S-source	Ca(NO3)2	500 mM	0.5 mM
	MgSO4	160 mM	0.16 mM
	KCl	670 mM	0.67 mM
	Na2CO3	190 mM	0.19 mM
N-source	Urea	500 mM	0.5 mM
P-source	Na2Glycerophosphate	163 mM	0.16 mM
pH Regulator	HEPES	250 mM	2.5 mM
Trace minerals	Na2EDTA	4.47 mM	13.4 µM
	FeCl3	717 µM	2.15 µM
	MnCl2	414 µM	1.24 µM
	ZnCl2	73 µM	0.22 µM
	CoCl2	17 µM	0.05 µM
	Na2MoO4	38.8 µM	0.10 µM
Vitamins	Thiamine (Vitamin B1)	1 mg/mL	1 µg/mL
	Biotin (Vitamin B7)	2.5 µg/mL	0.25 ng/mL
	Cyanocobalamin (Vitamin B12)	1.5 µg/mL	0.15 ng/mL

Description of the SVM Medium compounds and their final concentration.

Molar Composition

- [M]**2.2 mM** Na^+
- [M]**670 µM** K^+
- [M]**160 µM** Mg^{2+}
- [M]**500 µM** Ca^{2+}
- [M]**679 µM** Cl^-
- [M]**160 µM** SO_4^{2-}
- [M]**2.5 mM** $[HEPES]^-$
- [M]**1 mM** NO_3^-
- [M]**0.5 mM** Urea
- [M]**0.16 mM** $[Glycerophosphate]^{2-}$
- [M]**13 µM** $EDTA^{4-}$
- [M]**2.2 µM** Fe^{2+}
- [M]**0.22 µM** Zn^{2+}

- [M]**1.2 µM** Mn^{2+}
- [M]**0.1 µM** MoO_4^{2-}
- [M]**0.05 µM** Co^{2+}

MATERIALS TEXT

Required Materials:

- Stock solutions (see below)
- NaOH
- 1 x 2 L Beaker
- 1 x 1 L Measuring cylinder
- 2 x 1 L Bottles

Stock solutions:

Macro minerals, N-source, and S-source

- [M]**500 mM** $Ca(NO_3)_2$
- [M]**160 mM** $MgSO_4$
- [M]**670 mM** KCl
- [M]**190 mM** Na_2CO_3

N-source

- [M]**500 mM** Urea

P-source

- [M]**163 mM** Na_2Glycerophosphate

pH Regulator

- [M]**250 mM** HEPES

Trace Minerals ("P IV Metal solution" 2X)

- [M]**4.47 mM** Na_2EDTA
- [M]**717 µM** $FeCl_3$
- [M]**414 µM** $MnCl_2$
- [M]**73 µM** $ZnCl_2$
- [M]**38.8 µM** Na_2MoO_4

Vitamins

- [M]**1 mg/mL** Thiamine (Vitamin B_1)
- [M]**2.5 µg/ml** Biotin (Vitamin B_7)
- [M]**1.5 µg/ml** Cyanocobalamin (Vitamin B_{12})

Storage and Shelf Live of Stock Solutions

Room temperature

- Na_2CO_3
- P IV Metal Solution

4 °C

- $Ca(NO_3)_2$
- $MgSO_4$
- KCl urea
- Phosphoglycerate HEPES
- Thiamine (wraped in foil)

-20 °C

- Biotin
- Cyanocobalamin (in dark e.g. a cryobox)

Additional Notes:

1. All stocks except **biotin** and **cyanocobalamin** should be made fresh at least every 2 months.
2. The **trace metal** stocks should be made fresh every month, and sooner if there is any problem with the cultures that cannot be explained.

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

The amounts in this protocol are shown for **1 L SVM** but the protocol can be scaled up or down as desired if more or less SVM is needed.

Adding Solvent

1 Add **950 ml Initial MilliQ water** to a 2L beaker.

Adding Macro minerals, N-source, and S-source

2 Add **1 ml Ca(NO3)2** .

3 Add **1 ml MgSO4** .

4 Add **1 ml KCl** .

5 Add **1 ml Na2CO3** .

Adding N-source

6 Add **1 ml Urea** .

Adding P-source

7 Add **1 ml Na2Glycerophosphate** .

Adding pH Regulator

8 Add **10 ml HEPES** .

Adding Trace Minerals

9 Add **3 ml "P IV Metal solution" 2X** .

Adjusting pH and final volume

10 Adjust the pH to pH **7.8** with NaOH.

Make sure this is done under mild mixing.

11 Adjust the final volume to **1000 ml** .

Autoclaving

12 Dispense the mixture as 500 ml batches into desired 1L bottles.

13 Autoclave the medium.

14 Keep the medium at **4 °C** until you are ready to use it.

Adding Vitamins (Just before use)

15

Always add vitamins fresh and keep the medium at **4 °C** .

Be sure to work under a laminar flow hood of this step.

Using pre-plugged **sterile** tips mix the following as a cocktail in a 1.5 ml **sterile** microcentrifuge:

- **1000 µl Thiamine (Vitamin B1)**
- **100 µl Biotin (Vitamin B7)**
- **100 µl Cyanocobalamin**

15.1 Add **1000 µl Thiamine (Vitamin B1)** .

15.2 Add **100 µl Biotin (Vitamin B7)** .

15.3 Add **100 µl Cyanocobalamin** .

15.4 Mix.

16 Add the vitamins to the SVM-vit.