Preparation PCRS11-Red Sea Medium

The RCC Team

Abstract

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Protocol

Preparation of Hepes-NaOH 1M Stock Solution (skip if already available)

Step 1.

To 250mL of H_20, add gradually 119.15g of Hepes. Adjust pH at 7.5 and complete the volume at 500mL. Store in refrigerator.

AMOUNT
500 ml Additional info: Water
AMOUNT
119.15 g Additional info: Hepes

Preparation of Na2-EDTA/FeCl3 Stock Solution (skip if already available)

Step 2.

- To 40mL of HCl 0.1N, add gradually 1.080g of $FeCl_3$.

- To 40mL of NaOH 0.1N, add gradually 1.488g of Na_2-EDTA.

Now mix both solutions and fill up to the final volume of 2L with sterile water.Store in refrigerator

AMOUNT
1.08 g Additional info: FeCl3
AMOUNT
40 ml Additional info: HCl 0.1N
AMOUNT
40 ml Additional info: NaOH 0.1N
AMOUNT
1.448 g Additional info: Na2-EDTA

Preparation of Sodium Phosphate Stock Solution (skip if already available)

Step 3.

Prepare two solutions:

- Monosodium dihydrogen phosphate (NaH_2PO_4) at 50mM (6g in 1L)

- Disodium hydrogen phosphate (Na_2HPO_4) at 50mM (3.55g in 500mL)

Make an equimolar mixture of this two solutions and adjust the pH at 7.5.

CONCENTRATION
0.05 Molarity (M) Additional info: NaH2PO4
CONCENTRATION
0.05 Molarity (M) Additional info: Na2HPO4

Preparation of Trace metals “Gaffron+Se” Stock Solution (skip if already available)

Step 4.

To 500mL of H_20, add gradually the following nutrients

Quantity (mg/L)	Compound Final	Concentration in media (nM)
186	Boric acid (H_3BO_3)	150
101	Manganese (II) Sulfate Monohydrate ($MnSO_4.H_2O$)	30
1.98	Sodium Tungstate dihydrate ($Na_2WO_4.2H_20$)	0.3
5.16	Ammonium molybdate tetrahydrate ($(NH_4)_6MO_7O_{24}.4H_2O$)	1.45
7.14	Potassium bromide (KBr)	3
4.98	Potassium iodide (KI)	1.5
17.25	Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$)	3
9.25	Cadium Nitrate ($Cd(NO_3)_2.4H_2O$)	1.5
8.76	Cobalt (II) Nitrate ($Co(NO_3)_2.6H_2O$)	1.5
7.5	Copper (II) Sulfate ($CuSO_4.5H_2O$)	1.5
7.1	Nickel Chloride ($NiCl_2.6H_2O$)	1.5
2.4	Chromium (III) Nitrate ($Cr(NO_3)_3.9H_2O$)	0.3
1.5	Vanadyl Sulfate Pentahydrate ($VOSO_4.5H_2O$)	0.3
28.4	Aluminium Potassium Sulfate ($KAl(SO_4)_2.12H_2O$)	3
3.3	Selenium (IV) Oxyde (SeO_2)	1.5

Complete the volume at 1L and store in refrigerator.

Preparation PCRS11-Red Sea medium

Step 5.

To 1L of H2O, add 33,33g of Red Sea Salt. Dissolve by shake (20min on agitator)

Preparation PCRS11-Red Sea medium

Step 6.

Heat seawater during 20min at 100°C.

TEMPERATURE

100 °C Additional info:

Preparation PCRS11-Red Sea medium

Step 7.

Under the hood, to water, add these nutrients beforehand autoclaved (except vitamin):

Quantity	Compound	Final Concentration
1.0 mL	Hepes-NaOH 1M (pH 7.5) (See receipe below)	1mM
1.0 mL	Na_2-EDTA/$FeCl_3$ (See recipe above)	8µM
1.0 mL	Sodium Phosphate ($NaPO_4$) 50mM (pH 7,5) (See recipe above)	50 µM
1.0 mL	Ammonium Sulfate 400mM (NH4)2-SO4	400µM
0,1 mL	Trace metals “Gaffron+Se” (See recipe above)	
0.1 mL	Cyanocobalamin 10mg/L (Vit. B12)	1µg/L

Filter the medium on 0.2 microns.