Z8 Medium with salt water (25‰)

Standard medium for cyanobacteria growth.

PROTOCOL Z8 25 LIQUID

- Sterilize all glass material and/or use sterile plastic material;
- Use ultrapure water;
- When the distilled or deionized water is autoclaved, add 25g NaCl/L;
- Prepare the solutions in volumetric flasks;
- Autoclave separately the A,B, Fe-EDTA and micronutrients solutions;
- Prepare medium in the flow chamber under aseptic conditions;
- The solutions should be at room temperature when added to prevent precipitation;
- In the end stock solutions should be stored in the refrigerator. After cooling, add Vitamin B12 (final concentration in the medium, 10 µg/L). Defrost stock solution (1000x concentrated) and in the flow chamber (aseptic conditions), add the vitamin to the medium through vitamin filter-sterilized (0,2 µm).

Add the solutions to water according to the following concentrations:

Solution A	10 ml/L
Solution B	10 ml/L
Fe-EDTA Solution	10 ml/L
Micronutrients Solution	1 ml/L

Composition of stock Solutions

SOLUTION A

Reagent	Name	g/L
$NaNO_3$	Sodium Nitrate	46.7
$Ca(NO_3)_2.4H_2O$	Calcium Nitrate Tetrahydrate	5,9
$MgSO_4.7H_2O$	Magnesium Sulphate Heptahydrate	2.5

SOLUTION B

Reagent	Name	g/L
K_2HPO_4	Potassium Phosphate dibasic	3.1
$NaCO_3$	Sodium Carbonate	2.1

Fe-EDTA SOLUTION

Reagent	ml/L
$FeCl_3$	10
$NaCO_3$	9.5

MICRONUTRIENTS SOLUTION

Solution	ml/L
1 to 12	10
13 and 14	100

Composition of basic Solutions

$FeCl_3$ SOLUTION

Reagent	Name	100 ml
$FeCl_3.6H_2O$	Ferric Chloride	2.8 g
HCl (0.1 N)	Hydrochloric Acid	100 ml

EDTA-Na SOLUTION

Reagent	Name	100 ml
EDTA	Ethylenediamine Tetraacetic Acid	3.9 g
NaOH (0.1 N)	Sodium Hydroxide Solution	100 ml

SOLUTION 1 to 14

Nº	Reagent	Name	g/L	g/100ml
1	$Na_2WO_4.2H_2O$	Sodium Tungstate	0.33	0.033
2	$(NH_4)_6Mo_7O_{24}.2H_2O$	Ammonium Heptamolybdate	0.88	0.088
3	KBr	Potassium Bromide	1.2	0.12
4	KI	Potassium Iodide	0.83	0.083
5	$ZnSO_4.7H_2O$	Zinc Sulfate	2.87	0.287
6	$Cd(NO_3).4H_2O$	Cadium Nitrate	1.55	0.155
7	$Co(NO_3)_2.6H_2O$	Cobalt(II) nitrate	1.46	0.146
8	$CuSO_4.5H_2O$	Cupric Sulfate	1.25	0.125
9	$NiSO_4(NH_4)_2SO_4.6H_2O$	Ammonium Nickel Sulfate	1.98	0.198
10	$Cr(NO_3)_3.9H_2O$	Chromium(III) nitrate	0.41	0.041
11	V_2O_5	Vanadium (V) oxide	0.089	0.0089
12	$AlK(SO_4)_2.12H_2O$	Potassium Aluminium Sulfate	9.48	0.948
13	H_3BO_3	Boric Acid	3.1	0.31
14	$MnSo_4.4H_2O$	Manganese Sulfate	2.23	0.223

- Use ultrapure water with 1-1,5g agar per 100mL of water (1-1,5% w/v);
- Solutions should be at room temperature when added to prevent precipitation and water temperature with agar should not fall below 55-60 ° C, to prevent the agar polymerization;
- Prepare medium in the flow chamber under aseptic conditions;
- In the end stock solutions should be stored in the refrigerator.

Add the solutions to water according to the following concentrations:

Solution A	10 ml/L
Solution B	10 ml/L
Fe-EDTA Solution	10 ml/L
Micronutrients Solution	1 ml/L

Note: Stock solutions are the same as in liquid Z8 medium (see above).

- Gently shake after adding the stock solutions to prevent the formation of air bubbles
- Spread the semi-open plates in the flow chamber, distribute the medium by the plates, and wait until solidification (+/- 15/20 min).
- Close and invert the plates and, eventually, seal with parafilm. The set of plates should be sealed (eg: using the same bag containing the plates and that should not have left the flow chamber...) and preferably kept in the refrigerator until further use. At this time, open the bag with the plates in the flow chamber. It is possible that the plates have some water. If is that the case, partially open the plate(s) to use and wait until dry with the air of the flow chamber.

References:

Kótai, J. 1972. Instructions for preparation of modified nutrient solution Z8 for algae