OCT 17, 2023

DOI:
dx.doi.org/10.17504/protocols.io.n92ldzyrxv5b/v1

Protocol Citation: Estelle Bigeard 2023. F/2 medium at 27 PSU of salinity from Sea Red salts. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldzyrxv5b/v1

Protocol status: Working
We use this protocol and it's working

Created: Jan 07, 2022

Last Modified: Oct 17, 2023

PROTOCOL integer ID: 56659

Funders Acknowledgement:
ANR AQUARADOX, PARALEX, EPHEMER
Grant ID: SU & CNRS

F/2 medium at 27 PSU of salinity from Sea Red salts

Estelle
Bigeard[1]

[1]Station Biologique de Roscoff, France

Ecology of Marine Plankton (ECOMAP) team - Roscoff | Roscoff Culture Collection | 1 more workspace

Estelle Bigeard
Station Biologique de Roscoff, France, UMR7144 CNRS Sorbonne...

ABSTRACT

F/2 medium (Guillard's Marine Water Enrichment Solution) is used to maintain in culture microalgae (for example, dinoflagellates) and their marine parasites (Alveolates, Syndiniales & perkinsids, Fungi *Dinomyces*).

Most of these strains have been sampled from estuaries, at a 27 PSU of salinity. Adding soil extract is sometimes necessary to increase their growth. Since 2007, Roscoff Parasites Culture Collection was maintained in this medium.

In order to maintain the *Amoebophrya* Culture Collection with a medium which would be stable over time, we tested then validated since October 2022 the use of the Red Sea salt to prepare F/2 medium at a salinity of 27 PSU.

MATERIALS

Equipments

Autoclave
Laminar flow cabinet (or biosafety cabinet)
Stainless Steel Filter Holder
Peristaltic pump

Materials

RED SEA Salt 22 kg - Sel pour aquarium – Available on the website « Aqua Store » - Ref R11065
https://www.aqua-store.fr/sel-aquarium-eau-de-mer/1238-red-sea-salt-22-kilos-livraison-incluse-7290100771990.html

Guillard's (F/2) Marine Water Enrichment Solution 50 ×, liquid, plant cell culture tested - Merck Millipore (Sigma) - G9903

Stericup® Filter Unit - Merck Millipore - SCGVU10RE
or Filter Unit PES 0,2µm 1000mL – Nalgene – 567-0020
or 500 ml rapid-flow sterile bottle top filter with 0.2µ PES Membrane (Nalgene) - Thermo Fisher Scientific - 595-4520 linked to a DURAN 1000ml glass bottle (use the same clean bottle)

10L Polycarbonate carboys (Nalgene) - ThermoScientific - 2251-0020

1L Polycarbonate bottle (Nalgene) - ThermoScientific - 2015-1000

Pipette

Flow Cytometry

SYBR® Green I Nucleic Acid Gel Stain - Euromedex - 17590-1mL

BEFORE START INSTRUCTIONS

Red Sea Salt

Red Sea Salt is naturally harvested directly from the waters of the Red Sea. Natural seawater contains over 70 chemical elements. Most of them influence water parameters, but only a few play a significant role in its overall chemical stability.
The Red Sea contains moderately high yet balanced levels of Fundamental Elements (Calcium, Magnesium, Bicarbonates). These three "Fundamental Elements" have a major impact on the stability of pH, alkalinity, and the ionic strength of seawater. There are no nitrates or phosphates (algae nutrients), no toxic levels of heavy metals, nor chemical binders.

More informations to the link : https://g1.redseafish.com/fr/red-sea-salts/red-sea-salt/

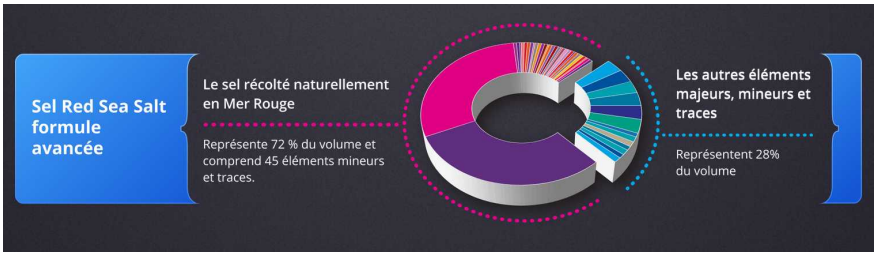

Figure 1 : Red Sea Salt Composition

Preparation of the (Natural, Controled) Seawater Solution

1 **Be careful** : Always use clean and non-toxic utensils for mixing.

Note: Due to possible salt stratification during transport, the entire salt should be mixed before sampling and dissolve it into water.

Use Milli-Q water as distilled water may content some trace elements such as Ca, Mg, HCO3.
Check the salinity of the mixed seawater using a specific seawater refractometer or a conductivity meter. Ensure that the measuring equipment is properly calibrated before its use.
Transparent containers are used as opaque containers cannot be autoclaved at 121°C.

Salinity	pH	Alkalinity (°dKH)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	Dose (g/L)
31.0 ppt	8,2-8,4	6,8-7,2	365-385	1090-1150	330-350	33,4
33,5 ppt	8,2-8,4	7,3-7,7	390-410	1170-1230	360-380	36,0
35,5 ppt	8,2-8,4	7,8-8,2	420-440	1250-1310	380-400	38,3

Figure 2 : Composition and salinity of seawater based on concentration.

Seawater Salinity 27 PSU
To obtain seawater with a salinity of 27 PSU using Red Sea Salt, weigh 294g of salt and transfer it to a 10L Nalgene container.
Add 10 liters of Milli-Q water at room temperature.
Shake the carboy.
Autoclave at 121°C for 20 minutes.

Comment : There is no need to mix before autoclaving until complete dissolution. Heating will dissolve salts, even if salts will

precipitate again during cooling.

Preparation of Culture Medium under Sterile Conditions

2 Think to thaw F/2 medium (Stored at -20°C) at 4°C.

Add 20mL of F/2 medium per liter of seawater at least the day before filtration, up to 3 days in advance.

Perform a sterile filtration using a 0.22µm filter unit, up to a maximum of 5L.
Check the salinity using a salinity probe or another suitable device.
Store at room temperature.

Sterility control using Flow Cytometry

3 Collect a sub-sampleof 1 mL from 2 or 3 1L Nalgene culture bottles in a cytometry tube.
Add 10µl of glutaraldehyde (25% grade) followed by 1% final concentration of SyBr Green for the DNA staining, wait 5 minutes at dark.
Bacteria population can be estimated using a flow cytometer, based on their FSC and Green fluorescence under a 488 laser excitation.

If bacteria are detected, the medium should be filtered and tested again.