f/2 Medium Derivatives and f/2 Agar

Black Sea Medium: For brackish water organisms (16 psu, half-strength nutrients). Combine 500 mL f/2 medium and 500 mL dH2O. Autoclave.

h/2 Medium: For ammonia loving organisms. Add 1mL of 500mM NH_4Cl per liter of f/2 medium to give a final concentration of 500uM ammonia. Autoclave.

f/2 agar: Prepare 1 liter of f/2 medium and dissolve 9g Bacto-agar (heat and mix). For test tubes, dispense dissolved agar medium into tubes, autoclave, and then cool with tubes slanted at an angle. For Petri plates, autoclave in a flask, cool almost to the gelling point, and then aseptically dispense into sterile Petri plates. Note: Agar can be added to other media (e.g., f/50 agar), and agar concentration can be varied to produce softer or firmer substrates.

f/2-Si: Prepare as for f/2 medium but omit Na2SiO 3 · 9H2O. This is preferred over f/2 medium for organisms with no silica requirement because less precipitation forms.

f/2 + Se: Extra silicon and selenium are beneficial to several diatom strains. Prepare 1 L of f/2 medium but use 2 mL of silicate stock, then add 1.0 mL of selenium stock solution (1.29 mg H2SeO 3 /L distilled H2O). Autoclave.

f/2 (11 psu): For brackish water organisms. Mix 650 mL distilled H2O and 350 mL filtered seawater. Add f/2 medium nutrients and autoclave.

f/2-Si (24 psu): Mix 750 mL distilled H2O and 250 mL filtered seawater. Prepare as for f/2 medium but omit Na2SiO 3 · 9H2O.

f/4: Add 500 mL f/2 medium to 500 mL filtered seawater, then autoclave.

f/4-Si: Autoclave 1 L of filtered seawater. When cool, aseptically add f/2-Si nutrients at half concentration (i.e., 0.5 mL).

f/20-Si: Autoclave 1 L of filtered seawater. When cool, aseptically add f/2-Si nutrients at one tenth concentration (i.e., 100uL).

f/50-Si: This is more than a 1/25 dilution of f/2-Si medium. We autoclave 1 L of seawater in a Teflon-lined bottle. Wait for the autoclaved seawater to cool to room temperature (important). Aseptically add 40 uL of sterile f/2 nutrients (20 uL of vitamins).

f/50-Si + CCMP1320 as food: Prepare f/50 and aseptically add 50uL of healthy, moderately dense culture of CCMP1320.

f/2m: To 1L f/2 medium add 1 g methylamine · HCl, mix until dissolved and autoclave. This medium is used to test for contamination by methylaminotrophic bacteria.

f/2p: To 1 L f/2 medium, add 1 g Bacto-peptone, mix until dissolves and autoclave. This medium is used to test for contamination by non- methylaminotrophic bacteria and fungi.

f/2pm: To 1L f/2 medium add 1 g Bacto-peptone and 1 g methylamine · HCl, mix until dissolved and autoclave. This general medium is used to test for contamination by bacteria and fungi.

f/2 + NPM: Add f/2 nutrients to 900 mL of seawater and autoclave. After cooling, aseptically add 100 mL of organic stock solution (see below). Dispense aseptically into test tubes.

Prov Add 15mL of a 1:50 dilution of Alkaline Soil Extract for a final volume of 1L of f/2-Si medium (see recipe for soil extract below)

Organics Stock Solution

(modified from Guillard 1960)

To 900 mL dH_2O add:

Quantity	Compound
1 g	sodium acetate
6 g	glucose
3 g	(di-) sodium succinate · $6H_2O$
4 g	neopeptone
1 g	Bacto-tryptone
100 mg	yeast extract

Bring up to 1 L with dH_2O. Dispense in small aliquots and autoclave.

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Alkaline Soil Extract

(Provasoli 1957)

Combine two parts dH_2O with one part rich organic garden soil (containing no recent applications of chemical fertilizer or pesticides). Add 2-3 g NaOH/liter. Autoclave for 2 hours, cool and filter. This concentrated extract is then diluted 5:1 with dH_2O to make the final working stock.

References

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