Bigelow | National Center for Marine Algae and Microbiota

L1 Derivatives

When the basic L1 medium does not accommodate the growth needs of specific strains, alterations and additions can be made as follows:

Black Sea Medium: For brackish water organisms (16 psu, half-strength nutrients). Combine 500 mL L1 medium and 500 mL dH_2O. Autoclave.

L1 (11): For brackish water organisms. Mix 650 mL distilled H2O and 350 mL filtered seawater. Add L1 medium nutrients and autoclave.

L1 (24): For brackish water organisms. Mix 750 mL distilled H2O and 250 mL filtered seawater. Prepare as for L1 medium but omit Na2SiO 3 · 9H2O.

h/2 Medium: For ammonia loving organisms. Add 1mL of 500mM NH_4Cl per liter of L1 medium to give a final concentration of 500uM ammonia. Autoclave.

L1+ NH_4Cl: For organisms do not love ammonia quite as much. Prepare as for L1 medium but omit the silica and add 300uL of a 500mM NH_4Cl per liter of L1 medium to give a final concentration of 166uM ammonia. Autoclave.

L1 agar: To grow algae on agar, prepare 1 liter of L1 medium and dissolve 9g 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:** The agar concentration can be varied to produce softer or firmer substrates.

L1 - Si: Prepare as for L1 medium but omit $Na_2SiO_3 \bullet 9H_2O$. This is preferred over L1 medium for organisms with no silica requirement because less precipitation forms.

L1 + Se: Extra silicon and selenium are beneficial to some diatom species. Prepare 1 L of L1 medium but use 2 mL of silicate stock, then add 1.0 mL of selenium stock solution (1.29 mg H_2SeO_3 /L distilled H_2O). Autoclave.

L/10-Si: for more fastidious organisms**.** Autoclave 1 L of filtered seawater. When cool, aseptically add L1-Si nutrients at one-tenth concentration (i.e., 100uL per L).

L1/25-Si: for even more delicate organisms. This is more than a 1/25 L1-Si medium. 1 L of seawater is autoclaved in a Teflon-lined bottle. After the autoclaved seawater cools to room temperature, L1 nutrients are added, aseptically, at one 25th concentration of L1 – Si (i.e., 40uL per L). We omit the silica. Check the pH after the additions and be sure that it is between 7.8-8.2. We find that it is usually too basic. Adjust with sterile HCl or NaOH as necessary.

L1m: This medium is used to test for contamination by methylaminotrophic bacteria.To 1L of L1 medium, add 1 g methylamine • HCl, mix until dissolved and autoclave.

L1p: This medium is used to test for contamination by non- methylaminotrophic bacteria and fungi.It can also be used by organisms that require an organic carbon source. To 1 L of L1 medium, add 1 g Bacto-peptone, mix until dissolves and autoclave.

L1pm: This general medium is used to test for contamination by bacteria and fungi.To 1L of L1 medium add 1 g Bacto-peptone and 1 g methylamine · HCl, mix until dissolved and autoclave.

L1 + NPM: For organisms requiring an organic carbon source**.** Add L1 nutrients to 900 mL of seawater and autoclave. After cooling, aseptically add 100 mL of organic stock solution (for recipe, see below). Dispense aseptically into test tubes. Or, you can add sterile NPM to each tube aseptically. We use an approximately 10% solution of NPM in L1 medium.

Prov: Add 20 mL of a 1:50 dilution of Alkaline Soil Extract for a final volume of 1L of L1-Si medium (see soil extract recipe below). At NCMA, we use the term Prov whether using a L1 base or an f/2 base.

Prov50, Prov100: see Prov50 medium page

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.

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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