

Prov50 Medium (and Prov 100)

(Provasoli & Guillard, unpublished)

The NCMA uses this seawater medium for organisms that grow better with (or require) soil-water extract and/or ammonia. The medium is similar to other soil-water extract media, such as Provasoli's ES medium (Provasoli 1968), Plymouth Laboratory's Erdschreiber Medium (Tompkins *et al.* 1995), or Sweeney's General Purpose Medium (Sweeney *et al.* 1959, Loeblich 1975) but has an ammonia addition. The method for preparing the alkaline soil water extract was first published by Provasoli *et al.* (1957), and he recommends using a higher concentration of the concentrated extract (e.g., 250 to 500 mL of concentrate per liter). The NCMA has slightly reduced the concentration (1 mL of stock = 200 mL of concentrate). **These media can be made with L1 trace metals as outlined below (used currently within NCMA collection), or using f/2 trace metals (see f/2 medium page for recipe).**

To prepare, begin with 950 mL of filtered natural seawater and add the following components. Recipes for the soil extract, trace element and vitamin solutions are provided below. Bring the final volume to 1 liter with filtered natural seawater. Autoclave.

| Component | Stock Solution | Quantity | Molar Concentration in Final Medium |
|---|--|----------|-------------------------------------|
| NaNO ₃ | 75 g/L dH ₂ O | 1 mL | 8.82 x 10 ⁻⁴ M |
| NH ₄ Cl | 2.67 g L ⁻¹ dH ₂ O | 1 mL | 5.00 x 10 ⁻⁵ M |
| NaH ₂ PO ₄ H ₂ O | 5 g/L dH ₂ O | 1 mL | 3.62 x 10 ⁻⁵ M |
| soil extract solution | (see recipe below) | 1 mL | --- |
| trace metal solution | (see recipe below) | 1 mL | --- |
| vitamin solution | (see recipe below) | 0.5 mL | --- |

Alkaline Soil Extract

(Provasoli *et al.* 1957)

Combine two parts dH₂O 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 dH₂O to make the final working stock and autoclaved.

L1 Trace Element Solution

To 950 mL dH₂O add the following components and bring final volume to 1 liter with dH₂O. Autoclave.

Na₂EDTA · 2H₂O should be added first and brought to pH 8.0-8.4 to assure it is fully dissolved. FeCl₃ · 6H₂O should be added next assure it is also fully dissolved prior to the addition of the remaining components.

| Component | Stock Solution | Quantity | Molar Concentration in Final PMedium |
|--|--|----------|--------------------------------------|
| Na ₂ EDTA · 2H ₂ O | --- | 4.36 g | 1.17 x 10 ⁻⁵ M |
| FeCl ₃ · 6H ₂ O | --- | 3.15 g | 1.17 x 10 ⁻⁵ M |
| MnCl ₂ ·4 H ₂ O | 178.10 g L ⁻¹ dH ₂ O | 1 mL | 9.00 x 10 ⁻⁷ M |
| ZnSO ₄ · 7H ₂ O | 23.00 g L ⁻¹ dH ₂ O | 1 mL | 8.00 x 10 ⁻⁸ M |
| CoCl ₂ · 6H ₂ O | 11.90 g L ⁻¹ dH ₂ O | 1 mL | 5.00 x 10 ⁻⁸ M |
| CuSO ₄ · 5H ₂ O | 2.50 g L ⁻¹ dH ₂ O | 1 mL | 1.00 x 10 ⁻⁸ M |
| Na ₂ MoO ₄ · 2H ₂ O | 19.9 g L ⁻¹ dH ₂ O | 1 mL | 8.22 x 10 ⁻⁸ M |
| H ₂ SeO ₃ | 1.29 g L ⁻¹ dH ₂ O | 1 mL | 1.00 x 10 ⁻⁸ M |
| NiSO ₄ · 6H ₂ O | 2.63 g L ⁻¹ dH ₂ O | 1 mL | 1.00 x 10 ⁻⁸ M |
| Na ₃ VO ₄ | 1.84 g L ⁻¹ dH ₂ O | 1 mL | 1.00 x 10 ⁻⁸ M |
| K ₂ CrO ₄ | 1.94 g L ⁻¹ dH ₂ O | 1 mL | 1.00 x 10 ⁻⁸ M |

f/2 Vitamin Solution

First, prepare primary stock solutions. To prepare final vitamin solution, begin with 950 mL of dH₂O, dissolve the thiamine, add the amounts of the primary stocks as indicated in the quantity column below, and bring final volume to 1 liter with dH₂O. At the NCMA we autoclave to sterilize. Store in refrigerator or freezer.

| Component | Primary Stock Solution | Quantity | Molar Concentration in Final Medium |
|--|---------------------------|----------|-------------------------------------|
| thiamine HCl (vit. B ₁) | --- | 200 mg | 2.96 x 10 ⁻⁷ M |
| biotin (vit. H) | 0.1g/L dH ₂ O | 10 mL | 2.05 x 10 ⁻⁹ M |
| cyanocobalamin (vit. B ₁₂) | 1.0 g/L dH ₂ O | 1 mL | 3.69 x 10 ⁻¹⁰ M |

Prov50 derivatives

Prov100: for ammonia loving organisms. Double the amount of ammonia used in Prov50 medium, adding 2mL of ammonia stock as defined to 1 liter of medium.

Prov50+ NPM: for organisms that require organic carbon. Add f/2 nutrients to 950 mL of seawater and autoclave. After cooling, aseptically add 50 mL of organic stock solution (see below). Dispense aseptically into test tubes. Or, sterile NPM may be added to each tube aseptically.

Prov50 + Rice: For heterotrophic organisms. The rice becomes food for bacteria that a eukaryote may eat, or it can supply organics as a source of nutrition. Add a grain of rice to a tube of unautoclaved Prov50 medium and then autoclave. This softens the rice and allows for maximum dissolution of the rice grain. If too much organics are released using this method, rice can be autoclaved separately and then added aseptically to sterile medium.

References

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