

## 28. PFENNIG'S MEDIUM I (modified 1988, for purple sulfur bacteria)

### Solution A:

CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.25	g
KH <sub>2</sub> PO <sub>4</sub>	1.70	g
NH <sub>4</sub> Cl	1.70	g
KCl	1.70	g
MgSO <sub>4</sub>	2.50	g
Distilled water	4000.00	ml

(For marine or estuarine isolates add 100.0 g NaCl to this solution and increase the MgSO<sub>4</sub> x 7 H<sub>2</sub>O to 15.0 g).

### Solution B:

Distilled water	860.00	ml
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Autoclave in a cotton-stoppered Erlenmeyer flask and cool to room temperature under an atmosphere of N<sub>2</sub> in an anaerobic jar.

### Solution C:

Vitamin B <sub>12</sub> solution (0.002% in H <sub>2</sub> O)	5.00	ml
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Filter sterilize.

### Solution D:

Trace element solution (SL-12 B)	5.00	ml
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Autoclave at 121°C for 15 min.

### Solution E:

NaHCO <sub>3</sub>	7.50	g
H <sub>2</sub> O	100.00	ml

Bubble with CO<sub>2</sub> and, after saturation, filter sterilize under CO<sub>2</sub> pressure into sterile, gas-tight, 100 ml screw-cap bottle.

### Solution F:

Na <sub>2</sub> S x 9 H <sub>2</sub> O (10 g in 100 ml)	20.00	ml
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Prepare in a screw-cap bottle, bubble with N<sub>2</sub> to replace air, close tightly and autoclave.

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*Trace element solution SL-12 B:*

Distilled water	1000.00	ml
Na <sub>2</sub> -EDTA	3.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	1.10	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
MnCl <sub>2</sub> x 2 H <sub>2</sub> O	50.00	mg
ZnCl <sub>2</sub>	42.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	18.00	mg
H <sub>3</sub> BO <sub>3</sub>	300.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg

Adjust pH to 6.0.

Autoclave solution A for 45 min. in 5-litre special bottle or flask (with four openings at the top) at 121°C, together with a teflon-coated magnetic bar. In this 5-litre bottle, two openings for tubes are in the central, silicon rubber stopper; a short, gas-inlet tube with a sterile cotton filter; and an outlet tube for medium, which reaches the bottom of the vessel at one end and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. The other two openings have gas-tight screw caps; one of these openings is for the addition of sterile solutions and the other serves as a gas outlet.

After autoclaving cool solution A to room temperature under a N<sub>2</sub> atmosphere with a positive pressure of 0.05 - 0.1 atm (a manometer for low pressure will be required). Saturate the cold medium with CO<sub>2</sub> by magnetic stirring for 30 min. under a CO<sub>2</sub> atmosphere of 0.05 - 0.1 atm. Add solution B, C, D, E and F through one of the screw-cap openings against a stream of either N<sub>2</sub> gas or better, a mixture of 95% N<sub>2</sub> and 5% CO<sub>2</sub> while the medium is magnetically stirred.

Adjust the pH of the medium with sterile HCl or Na<sub>2</sub>CO<sub>3</sub> solution (2 mol/liter each) to pH 7.3. Distribute the medium aseptically through the medium outlet tube into sterile, 100 ml bottles (with metal caps and autoclavable rubber seals) using the positive gas pressure (0.05 - 0.1 atm) of the N<sub>2</sub>/CO<sub>2</sub> gas mixture: Leave a small air bubble in each bottle to meet possible pressure changes. The tightly sealed, screw-cap bottles can be stored for several weeks or months in the dark. During the first 24 h, the iron of the medium precipitates in the form of black flocks. No other sediment should arise in the otherwise clear medium. Incubate in the light using a tungsten lamp. Feed periodically with neutralized solution of sodium sulfide (see medium 27) to replenish sulfide and with other supplement solutions (see Ref. 3365).