

29. PFENNIG'S MEDIUM II**Solution A:**

CaCl ₂ x 2 H ₂ O	0.25	g
Yeast extrakt	0.25	g
Distilled water	460.00	ml

Fill 10x 46ml in 100 ml screw-cap bottles. Bubble with N₂/CO₂ and autoclave 121°C 15 min.

(For marine or estuarine isolates add 100.0 g NaCl to this solution and increase the MgSO₄ x 7 H₂O to 15.0 g).

Solution B:

Na ₂ S x 9 H ₂ O	2.00	g
Distilled water	135.00	ml

Prepare in a screw-cap bottle, bubble with N₂ to replace air, close tightly and autoclave.

Solution C:

NaHCO ₃	1.50	g
H ₂ O	50.00	ml

Bubble with CO₂ and filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

Solution D

Resazurin (0,1%)	0.5	ml
Distilled water	450.00	ml

Autoclave in a cotton-stoppered Erlenmeyer flask with an outlet tube for medium, connected to a glass outlet at the bottom of the vessel 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.

Cool to room temperature under an atmosphere of N₂/CO₂ in an ice bath.

Solution E:

Ammonium chloride	0.35	g
Ammonium acetate	0.25	g
Pyruvic acid sodium salt	0.25	g
Dextrose	0.25	g
MgSO ₄ x7H ₂ O	0.50	g
KCL	0.35	g
KH ₂ PO ₄	0.35	g
Trace element solution SL-10 B	1.00	ml
Distilled water	25	ml

Filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

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Solution F:

Vitamin B ₁₂	0.01	g
Distilled water	100.00	ml
Filter sterilized		

Trace element solution SL-10 B:

Distilled water	1000.0	ml
HCl (25%)	7.7	ml
FeSO ₄ x 7 H ₂ O	1.5	g
ZnCl ₂	70.0	mg
MnCl ₂ x 4 H ₂ O	100.0	mg
H ₃ BO ₃	300.0	mg
CoCl ₂ x 6 H ₂ O	190.0	mg
CuCl ₂ x 2 H ₂ O	2.0	mg
NiCl ₂ x 6 H ₂ O	24.0	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.0	mg

Mix solution D, C and E. Bubble with CO₂ in an ice bath under sterile conditions. Fill 50 ml in each bottle of solution A. Before using add 4 ml solution B and 0.1 ml solution F.

Adjust the pH with filter-sterilised 1M Na₂CO₃ to 6.8 -7.1.

Fill in sterile, N₂ gassed screw-cap tubes under N₂ gas.

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. Feed periodically with neutralized 3% solution of sodium sulfide to replenish sulfide and with other supplement solutions (see Ref. 3365).

Neutralized sulfide solution:

Distilled water	100.00	ml
Na ₂ S x 9 H ₂ O	3.00	g

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 N H₂SO₄ drop-wise with a syringe without opening the bottle.

Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.