

## 29: PFENNIG'S MEDIUM II

This recipe contains strain-specific modifications for *Chlorobaculum thiosulfatophilum* DSM 249 \*

Final pH: 6.8 - 7.1

Final volume: 1000 ml

<b>Solution A</b>	460.00	ml
<b>Solution B</b>	40.00	ml
<b>Solution C</b>	50.00	ml
<b>Solution D</b>	450.00	ml
<b>Solution E</b>	26.00	ml
<b>Solution F</b>	10.00	ml

1. Mix solution D, C and E. Bubble with CO<sub>2</sub> in an ice bath under sterile conditions.
2. Fill 50 ml in each bottle of solution A. Before using add 4 ml solution B and 0.1 ml solution F.
3. Adjust the pH with filter-sterilised 1M Na<sub>2</sub>CO<sub>3</sub> to 6.8 -7.1.
4. Fill in sterile, N<sub>2</sub> gassed screw-cap tubes under N<sub>2</sub> gas.
5. 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).

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### **Solution A** (from medium 29)

CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.25	g
Yeast extract	0.25	g
Distilled water	460.00	ml

Fill 10x 46ml in 100 ml screw-cap bottles. Bubble with N<sub>2</sub>/CO<sub>2</sub> and autoclave 121°C 15 min.

### **Solution B** (from medium 29)

Distilled water	135.00	ml
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Prepare in a screw-cap bottle, bubble with N<sub>2</sub> to replace air, close tightly and autoclave.

### **Solution C** (from medium 29)

NaHCO <sub>3</sub>	1.50	g
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H <sub>2</sub> O	50.00	ml
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Bubble with CO<sub>2</sub> and filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

### **Solution D** (from medium 29)

Resazurin (0,1%)	0.50	ml
Distilled water	450.00	ml

1. 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.
2. Cool to room temperature under an atmosphere of N<sub>2</sub>/CO<sub>2</sub> in an ice bath.

### **Solution E** (from medium 29)

Ammonium chloride	0.35	g
Ammonium acetate	0.25	g
Pyruvic acid sodium salt	0.25	g
Dextrose	0.25	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
KCl	0.35	g
KH <sub>2</sub> PO <sub>4</sub>	0.35	g
<b>Trace element solution SL-10 B</b>	1.00	ml
Distilled water	25.00	ml

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

### **Solution F** (from medium 29)

Vitamin B <sub>12</sub>	0.01	g
Distilled water	100.00	ml

Filter sterilized

### **Neutralized sulfide solution 3% (w/v)** (from medium 28)

Na <sub>2</sub> S x 9 H <sub>2</sub> O	3.00	g
Distilled water	100.00	ml

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 M H<sub>2</sub>SO<sub>4</sub> drop-wise with a syringe without opening the bottle.

### Trace element solution SL-10 B

Distilled water	1000.00	ml
HCl (25%)	7.70	ml
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	300.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	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	36.00	mg