

## 28: PFENNIG'S MEDIUM I

This recipe contains strain-specific modifications for *Roseospira marina* DSM 15113 \*

Final pH: 7.1 - 7.3

Final volume: 1000 ml

<b>Solution A</b>	460.00	ml
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1. Aliquot Solution A into 100 mL screw-cap bottles, filled with 46 mL each. Bubble with N<sub>2</sub>/CO<sub>2</sub> and autoclave at 121°C for 15 min (as described below).

2. Prepare the following solutions (resazurin, bicarbonate and Pfennig's heterotrophic salts) and sterilize as given below.

<b>Resazurin solution</b>	450.00	ml
<b>Bicarbonate solution</b>	50.00	ml
<b>Pfennig's heterotrophic salts solution</b>	26.00	ml

3. Add bicarbonate solution and Pfennig's heterotrophic salts to the resazurin (complete volume, i.e. 50 mL bicarbonate solution and 26 mL Pfennig's heterotrophic salts solution). Bubble with CO<sub>2</sub> in an ice bath under sterile conditions.

4. Fill 50 ml of this mixture to each bottle of solution A (46 mL + 50 mL).

5. Before use, add 4 ml sulfide solution (1.5%) and 0.1 ml Vitamin B<sub>12</sub> solution to each 100 mL bottle.

<b>Sulfide solution, 1.5%</b>	40.00	ml/l
<b>Vitamin B<sub>12</sub> solution</b>	1.00	ml/l

6. Adjust the pH with filter-sterilised 1M Na<sub>2</sub>CO<sub>3</sub> to 7.1-7.3.

7. If needed, aliquot into sterile, N<sub>2</sub> gassed screw-cap tubes under N<sub>2</sub> gas.

8. 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.

9. Feed the actively growing culture periodically with neutralized 3% solution of sodium sulfide (use 1 -3 mL/100 mL depending on strain and cultivation stage) to replenish sulfide and with other supplement solutions (see Ref. 3365).

<b>Neutralized sulfide solution 3% (w/v)</b>	10.00	ml
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\* plus 2% NaCl plus 0,1% MgCl<sub>2</sub>·6H<sub>2</sub>O plus 0,15% Na-acetate, Na-thiosulfate, Yeast extract plus 5ml 0,01% Vitamine B<sub>12</sub>/l

**Solution A** (from medium 28)

CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.25	g
Yeast extract	0.25	g

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Distilled water	460.00	ml
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Aliquot Solution A into 100 mL screw-cap bottles, filled with 46 mL each. Bubble with N<sub>2</sub>/CO<sub>2</sub> and autoclave at 121°C for 15 min.

### **Sulfide solution, 1.5%** (from medium 28)

Na <sub>2</sub> S x 9 H <sub>2</sub> O	2.00	g
Distilled water	135.00	ml

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

### **Bicarbonate solution** (from medium 28)

NaHCO <sub>3</sub>	1.50	g
H <sub>2</sub> O	50.00	ml

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

### **Resazurin solution** (from medium 28)

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.

### **Pfennig's heterotrophic salts solution** (from medium 28)

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-12 B</b>	1.00	ml
Distilled water	25.00	ml

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

### **Vitamin B<sub>12</sub> solution** (from medium 28)

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

Filter sterilized

### Trace element solution SL-12 B (from medium 28)

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
Distilled water	1000.00	ml

Adjust pH to 6.0.

### 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.