28: PFENNIG'S MEDIUM I

This recipe contains strain-specific modifications for *Rhodospira trueperi* DSM 117282 *

Final pH: 7.1 - 7.3
Final volume: 1000 ml

Solution A	460.00	ml

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

2. Aliquot Solution A into 100 mL screw-cap bottles, filled with 46 mL each. Bubble with N_2/CO_2 and autoclave at 121°C for 15 min (as decribed below).

Resazurin solution	450.00	ml
Bicarbonate solution	50.00	ml
Pfennig's heterotrophic salts solution	26.00	ml
$MgCl_2$ x 6 H_2O	0.30	%

3. Add bicarbonate solution and Pfennig's heterotrophic salts to the resazurin (complete volumina, i.e. 50 mL bicarbonate solution and 26 mL Pfennig's heterotrophic salts solution). Bubble with CO_2 in an ice bath under sterile conditions.

NaCl	2.00	%

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_{12} solution to each 100 mL bottle.

Sulfide solution, 1.5%	40.00	ml/l
Vitamin B_{12} solution	1.00	ml/l
Vitamin solution A (1000 x stock)	0.25	ml
Vitamin solution B (1000 x stock)	0.25	ml

6. Adjust the pH with filter-sterilised 1M Na_2CO_3 to 7.1-7.3.

7. If needed, aliquot into sterile, N_2 gassed screw-cap tubes under N_2 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).

Neutralized sulfide solution 3% (w/v)	10.00	ml

* Medium 28 + 2 % NaCl+ Vitamine A+B Medium 1783 0,25ml/l+ 0,3% MgCl2x6H2O, used for Rhodospira trueperi

Solution A (from medium 28)

$CaCl_2$ x 2 H_2O	0.25	g
Yeast extract	0.25	g
Distilled water	460.00	ml

Aliquot Solution A into 100 mL screw-cap bottles, filled with 46 mL each. Bubble with N_2/CO_2 and autoclave at 121°C for 15 min.

Sulfide solution, 1.5% (from medium 28)

Na_2S x 9 H_2O	2.00	g
Distilled water	135.00	ml

Prepare in a screw-cap bottle, bubble with N_2 to replace air, close tightly and autoclave.

Bicarbonate solution (from medium 28)

$NaHCO_3$	1.50	g
H_2O	50.00	ml

Bubble with CO_2 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_2/CO_2 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
$MgSO_4$ x 7 H_2O	0.50	g
KCl	0.35	g
KH_2PO_4	0.35	g
Trace element solution SL-12 B	1.00	ml
Distilled water	25.00	ml

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

Vitamin B_{12} solution (from medium 28)

Vitamin B_{12}	0.01	g
Distilled water	100.00	ml

Filter sterilized

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

Na_2-EDTA	3.00	g
$FeSO_4$ x 7 H_2O	1.10	g
$CoCl_2$ x 6 H_2O	190.00	mg
$MnCl_2$ x 2 H_2O	50.00	mg
$ZnCl_2$	42.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	18.00	mg
H_3BO_3	300.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
Distilled water	1000.00	ml

Adjust pH to 6.0.

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

Na_2S x 9 H_2O	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_2SO_4 drop-wise with a syringe without opening the bottle.

Vitamin solution A (1000 x stock)* (from medium 1783)

Biotin	10.00	mg
Riboflavin	10.00	mg
Thiamine HCl	100.00	mg
Thiamine pyrophosphate	100.00	mg
L-Ascorbic acid	100.00	mg
Calcium pantothenate	100.00	mg
Folic acid	100.00	mg
Nicotinamide	100.00	mg
Nicotinic acid	100.00	mg
p-Aminobenzoic acid	100.00	mg
Pyridoxine hydrochloride	100.00	mg

Lipoic acid	100.00	mg
beta-NAD	100.00	mg
Potassium phosphate buffer (pH 7.2, 10 mM)	100.00	ml

1. Dissolve the vitamins in 10mM phosphate buffer, pH7.2.

2. Titrate with NaOH until vitamins are dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage.

Vitamin solution B (1000 x stock)* (from medium 1783)

Vitamin B_{12}	100.00	mg
Distilled water	100.00	ml

1. Dissolve the vitamin B_{12} in water.

2. Titrate with HCl until vitamin B_{12} is dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage.