

27. RHODOSPIRILLACEAE MEDIUM (modified)

Yeast extract	0.30	g
Ethanol	0.50	ml
Na ₂ -succinate	1.00	g
(NH ₄)-acetate	0.50	g
Fe(III) citrate solution (0.1% in H ₂ O)	5.00	ml
KH ₂ PO ₄	0.50	g
MgSO ₄ x 7 H ₂ O	0.40	g
NaCl	0.40	g
NH ₄ Cl	0.40	g
CaCl ₂ x 2 H ₂ O	0.05	g
Vitamin B ₁₂ solution (10 mg in 100 ml H ₂ O)	0.40	ml
Trace element solution SL-6 (see below)	1.00	ml
Distilled water	1050.00	ml

Adjust pH to 6.8.

Boil the medium under a stream of nitrogen gas for few minutes and distribute 45 ml medium into 50 ml screw-capped bottles (already flushed with nitrogen gas). Bubble each bottle with nitrogen gas and close immediately with a rubber septum and screw tight. Autoclave at 121°C for 15 min. Sterile syringes are used to inoculate and remove samples.

Incubate in the light using a tungsten lamp. For brown and other oxygen sensitive Rhodospirillaceae add 300 mg of L-cysteine (0.03% end concentration) to the boiling medium and readjust the pH to 6.8 or to the prepared medium in bottles inject neutralized sulfide solution (0.005 to 0.01% end concentration). The medium has been modified according to reference 3365.

Trace element solution SL-6:

ZnSO ₄ x 7 H ₂ O	0.10	g
MnCl ₂ x 4 H ₂ O	0.03	g
H ₃ BO ₃	0.30	g
CoCl ₂ x 6 H ₂ O	0.20	g
CuCl ₂ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.02	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
Distilled water	1000.00	ml

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Neutralized sulfide solution:

Distilled water	100.00	ml
Na ₂ S x 9 H ₂ O	1.50	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 7.3 by adding of sterile 2 M 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.