

87. CHLOROFLEXUS MEDIUM (modified)

Yeast extract	1.00	g
Glycyl-glycine	1.00	g
Na ₂ HPO ₄ x 2 H ₂ O	0.10	g
MgSO ₄ x 7 H ₂ O	0.10	g
KNO ₃	0.10	g
NaNO ₃	0.50	g
NaCl	0.10	g
CaCl ₂ x 2 H ₂ O	0.05	g
Fe(III) citrate solution (0.1 g in 100 ml H ₂ O)	5.00	ml
Trace element solution SL-6 (see medium 27)	1.00	ml
Distilled water	1050.00	ml

Adjust pH to 8.2.

Boil the medium under a stream of nitrogen gas for a few minutes and distribute 90 ml medium into 100 ml screw-capped bottles. Bubble each bottle with nitrogen gas and close immediately with a rubber septum and screw tight. Autoclave at 121°C for 15 min. After autoclaving inject 1.0 ml of neutralized sulfide solution (0.03% end concentration) to each bottle. This medium can be stored for several months. Incubate the culture at 50°C at a light intensity of 300 - 500 lux. For heavy cell suspension supplement periodically with sterile yeast extract solution (0.1% end concentration, for more details see Ref. 3365, 3366).

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 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.