

1667. HYPNOCYCLICUS MEDIUM

Na ₂ SO ₄	0.02	g
KH ₂ PO ₄	1.00	g
NH ₄ Cl	0.10	g
NaCl	20.00	g
MgCl ₂ x 6 H ₂ O	3.00	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
NaNO ₃	0.70	g
Yeast extract	1.00	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Vitamin solution (see medium 503)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, vitamins and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.2, if necessary.