

521. DESULFOMONILE MEDIUM

NH ₄ Cl	1.0	g
Na ₂ SO ₄	2.0	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	1.0	g
MgSO ₄ x 7 H ₂ O	1.0	g
CaCl ₂ x 2 H ₂ O	0.1	g
KH ₂ PO ₄	0.5	g
Trace element solution SL-10 (see medium 320)	1.0	ml
Selenite-tungstate solution (see medium 385)	1.0	ml
Yeast extract	1.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml
NaHCO ₃	1.0	g
Vitamin solution (see below)	10.0	ml
Na-pyruvate	2.5	g
Na ₂ S x 9 H ₂ O	0.1	g
Distilled water	1000.0	ml

Dissolve ingredients (except bicarbonate, vitamins, pyruvate and sulfide), then sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete the medium by adding vitamins, pyruvate and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Stock solutions of vitamins and pyruvate are sterilized by filtration. Adjust pH of the complete medium to 6.8 - 7.0.

Note: Prior to inoculation 10-20 mg/l sodium dithionite (added from a 5% w/v solution freshly prepared under N₂ and filter-sterilized) can be added to the medium to stimulate growth at the beginning.

Vitamin solution:

Pyridoxine-HCl	5.0	mg
Riboflavin	5.0	mg
Ca-pantothenate	5.0	mg
p-Aminobenzoate	5.0	mg
Thioctic acid	5.0	mg
Nicotinamide	50.0	mg
Biotin	5.0	mg
Folic acid	5.0	mg
Vitamin B ₁₂	5.0	mg
Haemin (dissolved in 0.1 N NaOH)	5.0	mg
1,4-Naphthoquinone (dissolved in 0.1 N NaOH)	20.0	mg
Distilled water	1000.0	ml