

480a. DESULFURELLA MEDIUM (BRACKISH WATER)

NaCl	10.00	g
NH ₄ Cl	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
KCl	0.33	g
KH ₂ PO ₄	0.33	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract	0.10	g
Na-acetate	5.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powder	10.00	g
NaHCO ₃	2.50	g
Vitamin solution (see medium 141)	10.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, bicarbonate, vitamins and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 5.9, dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and sterilize medium by heating in a boiling water bath for 2- 3 hours on each of 3 successive days. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to use adjust pH of complete medium to 6.8 - 7.0, if necessary.