

1526d. LIMISALSIVIBRIO (L21 LS) MEDIUM

NaCl	60.0	g
MgCl ₂ x 6 H ₂ O	6.0	g
KCl	1.5	g
Na ₂ SO ₄	1.0	g
NH ₄ Cl	1.0	g
CaCl ₂ x 2 H ₂ O	0.4	g
K ₂ HPO ₄	0.4	g
Modified Wolin's mineral solution (see medium 141)	10.0	ml
Na-resazurin solution (0.1% w/v)	0.5	ml
Na ₂ CO ₃	1.0	g
Na-acetate	1.6	g
Na ₂ -fumarate	6.4	g
Seven vitamins solution (see medium 503)	1.0	ml
Na ₂ S x 9 H ₂ O	0.5	g
Distilled water	1000.0	ml

Dissolve ingredients except carbonate, acetate, fumarate, vitamins, cysteine and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate, fumarate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas (fumarate and vitamins are sterilized by filtration) and carbonate 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.