

1233. CALDICOPROBACTER MEDIUM

NaCl	0.50	g
KCl	0.20	g
MgCl ₂ x 6 H ₂ O	0.18	g
NH ₄ Cl	0.10	g
HEPES (SIGMA)	4.80	g
Na ₂ PO ₄ x 7 H ₂ O	54.00	mg
Fe(NH ₄)SO ₄ x 6 H ₂ O solution (0.1% w/v)	4.00	ml
CaCl ₂ x 2 H ₂ O solution (0.1% w/v)	2.00	ml
MnCl ₂ x 4 H ₂ O solution (0.1% w/v)	1.00	ml
Yeast extract (OXOID)	5.00	g
Tryptone (BD Bacto)	2.50	g
Na-resazurin solution (0.1% w/v)	0.50	ml
L-Cysteine-HCl x H ₂ O	1.00	g
D-Glucose	5.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine and glucose), then sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Add solid cysteine and adjust pH to 7.3 – 7.5, then dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before inoculation, add glucose from a sterile anoxic stock solution prepared under 100% N₂ gas. Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) can be used to completely reduce the medium and may stimulate growth at the beginning.