

858. ANAEROBIC CHOLESTEROL MEDIUM**Solution A:**

MgSO ₄ x 7 H ₂ O	0.10	g
NH ₄ Cl	0.10	g
CaCl ₂	0.02	g
NaNO ₃	0.16	g
Distilled water	200.00	ml

Solution B:

KH ₂ PO ₄	1.00	g
Distilled water	100.00	ml

Solution C:

Trace elements SL-10 (see medium 320)

Solution D:

Vitamin solution (see medium 461)

Solution E:

Selenite-tungstate solution (see medium 385)

Solution F:

NaHCO ₃	8.40	g
Distilled water	100.00	ml

Prepare solution A, distribute appropriate amounts of cholesterol (e.g. 5 mg for 10 ml medium) and solution A in anaerobic tubes, and autoclave anaerobically under 80% N₂ + 20% CO₂. Solutions B and F are also autoclaved under these conditions. Solution C, D and E may be prepared aerobically. After cooling, add to 9 ml solution A: 500 µl sol. B, 500 µl sol. F, 25 µl (one drop) sol. C and E, each, and 50 µl sol. D under anaerobic conditions. Adjust pH to 7.0±0.2.