

336. OXOBACTER PFENNIGII MEDIUM**Solution A:**

Mineral solution (see medium 335)	50.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract	2.00	g
Na-vanillate	2.00	g
Rumen fluid, clarified (see medium 1310)	300.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	540.00	ml

Adjust pH to 6.9 before autoclaving.

Solution B:

NaHCO ₃	5.00	g
Distilled water	100.00	ml

Solution C:

Vitamin solution (see medium 503)	1.00	ml
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Solution D:

L-Cysteine-HCl x H ₂ O	0.24	g
Distilled water	10.00	ml

Solution E:

Na ₂ S x 9 H ₂ O	78.00	mg
Distilled water	1.00	ml

Sparge *solution A* with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, distribute under same gas atmosphere 8.9 ml medium per Hungate-type tube and autoclave. *Solution B* is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. *Solution C* is prepared under 100% N₂ gas atmosphere and sterilized by filtration. Autoclave *solutions D* and *E* under 100% N₂ gas. To complete the medium add appropriate amounts of the *solutions B* to *E* to the sterile *solution A*. Adjust pH of complete medium to 7.0 - 7.2, if necessary.