

**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	620.00	ml

Adjust pH to 6.9 before autoclaving.

**Solution B:**

Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Distilled water	30.00	ml

**Solution C:**

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

L-Cysteine-HCl x H <sub>2</sub> O	0.30	g
Distilled water	10.00	ml

**Solution E:**

Na <sub>2</sub> S x 9 H <sub>2</sub> O	90.00	mg
Distilled water	3.00	ml

Sparge *solution A* with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. *Solution C* is prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Autoclave *solutions D* and *E* under 100% N<sub>2</sub> 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.