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:
- Na₂CO₃ 1.50 g
- Distilled water 30.00 ml

Solution C:
- Vitamin solution (see medium 503) 1.00 ml

Solution D:
- L-Cysteine-HCl x H₂O 0.30 g
- Distilled water 10.00 ml

Solution E:
- Na₂S x 9 H₂O 90.00 mg
- Distilled water 3.00 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.