346. DESULFALLAS MEDIUM

Solution A:
- Na$_2$SO$_4$ 3.00 g
- KH$_2$PO$_4$ 0.20 g
- NH$_4$Cl 0.30 g
- CaCl$_2$ x 2 H$_2$O 0.15 g
- MgCl$_2$ x 6 H$_2$O 0.40 g
- NaCl 1.00 g
- Trace element solution SL-10 (see medium 320) 1.00 ml
- Na-resazurin solution (0.1% w/v) 0.50 ml
- Distilled water 965.00 ml

Solution B:
- NaHCO$_3$ 1.00 g
- Distilled water 20.00 ml

Solution C:
- Ethanol 1.00 ml
- Distilled water 10.00 ml

Solution D:
- Na-benzoate 0.70 g
- Distilled water 10.00 ml

Solution E:
- Rumen fluid, clarified (see medium 1310) 1.00 ml

Solution F:
- Vitamin solution (see medium 141) 10.00 ml

Solution G:
- Na-dithionite 25.00 mg
- Distilled water 1.00 ml

Solution H:
- Na$_2$S x 9 H$_2$O 50.00 mg
- Distilled water 1.70 ml

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Sparge solution A with 100% N₂ gas for 30 - 45 min to make it anoxic. Thereafter, dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Anoxic stock solutions C, D, E, F, G and H are prepared separately under 100% N₂ gas atmosphere. Filter sterilize solutions D, F and G. To complete the medium appropriate amounts of solutions B to H are added to the sterile solution A in the sequence as indicated. Adjust pH of the complete medium to 7.2 - 7.5, if necessary.