829a: DESULFUROBACTERIUM ATLANTICUM MEDIUM

NaCl	30.00	g
$MgCl_2$ x 6 H_2O	3.00	g
$CaCl_2$ x 2 H_2O	0.15	g
KCl	0.50	g
NH_4Cl	0.50	g
MES [2-(N-morpholino) ethane sulfonic acid]	1.95	g
KH_2PO_4	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
$Na_2S_2O_3$ x 5 H_2O	2.50	g
Neutralized sulfide solution 3% (w/v)	20.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except thiosulfate and sodium sulfide), adjust pH to 6.0, boil medium for 1 min, then cool to room temperature under 80% H_2 and 20% CO_2 gas atmosphere. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add sodium thiosulfate from an anoxic stock solution prepared under 100% N_2 gas and sterilized by filtration. Reduce medium with a sterile, neutralized solution of sodium sulfide prepared under 100% N_2 gas . Adjust pH of complete medium to 6.0, if necessary.

2. After inoculation pressurize cultivation vessels to 2 bar overpressure using sterile 80% H_2 and 20% CO_2 gas mixture.

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na_2S x 9 H_2O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.