

## 358a. ACIDIANUS MEDIUM (ANAEROBIC)

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.28	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.07	g
FeCl <sub>3</sub> x 6 H <sub>2</sub> O	0.02	g
Allen's trace element solution (see medium 88)	10.00	ml
Sulfur, powder	5.00	g
Yeast extract (OXOID)	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients, except sulfur and yeast extract, bring medium to the boil, then cool to room temperature under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and adjust pH to 2.5 using 10 N H<sub>2</sub>SO<sub>4</sub>. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 100 ml serum bottles) containing already the appropriate amount of sulfur. For sterilization sealed bottles with medium are heated in a boiling water bath for 2 - 3 h on each of 3 successive days. Add yeast extract from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere. Pressurize inoculated bottles to 1 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

*Note: Inoculate with 5% (w/v) culture. Incubate without shaking.*

For [DSM 3772](#) reduce amount of yeast extract to 0.02 g/l.

For [DSM 6296](#) reduce amount of yeast extract to 0.20 g/l and adjust pH to 2.5 – 3.0. Pressurize inoculated bottles to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

For [DSM 6334](#) reduce amount of yeast extract to 0.20 g/l. Pressurize inoculated bottles to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.