

**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
<b>Allen's trace element solution</b>	10.00	ml
Sulfur (powder)	5.00	g
Yeast extract (OXOID)	0.50	g
Distilled water	1000.00	ml

1. 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.

2. Pressurize inoculated bottles to 1 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

3. 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.

**Allen's trace element solution** (from medium 88)

MnCl <sub>2</sub> x 4 H <sub>2</sub> O	180.00	mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> x 10 H <sub>2</sub> O	450.00	mg
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	22.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	5.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	3.00	mg
VOSO <sub>4</sub> x 2 H <sub>2</sub> O	3.00	mg
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	1.00	mg
Distilled water	1000.00	ml

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Adjust pH of final solution to 2 with 1 N HCl.