

358a: ACIDIANUS MEDIUM (ANAEROBIC)

This recipe contains strain-specific modifications for *Stygiolobus azoricus* DSM 6296 *

Final pH: * 2.5 - 3.0

Final volume: 1010 ml

(NH ₄) ₂ SO ₄	1.30	g
KH ₂ PO ₄	0.28	g
MgSO ₄ x 7 H ₂ O	0.25	g
CaCl ₂ x 2 H ₂ O	0.07	g
FeCl ₃ x 6 H ₂ O	0.02	g
Allen's trace element solution	10.00	ml
Sulfur (powder)	5.00	g
Yeast extract (OXOID)	0.20	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% H₂ and 20% CO₂ gas mixture and adjust pH to 2.5 using 10 N H₂SO₄. 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% N₂ gas atmosphere.

2. Pressurize inoculated bottles to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

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

* 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% H₂ and 20% CO₂ gas mixture.

Allen's trace element solution (from medium 88)

MnCl ₂ x 4 H ₂ O	180.00	mg
Na ₂ B ₄ O ₇ x 10 H ₂ O	450.00	mg
ZnSO ₄ x 7 H ₂ O	22.00	mg
CuCl ₂ x 2 H ₂ O	5.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	3.00	mg
VO ₂ SO ₄ x 2 H ₂ O	3.00	mg
CoSO ₄ x 7 H ₂ O	1.00	mg
Distilled water	1000.00	ml

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