

## 358a. ACIDIANUS MEDIUM (ANAEROBIC)

$(\text{NH}_4)_2\text{SO}_4$	1.30	g
$\text{KH}_2\text{PO}_4$	0.28	g
$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$	0.25	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	0.07	g
$\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$	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%  $\text{H}_2$  and 20%  $\text{CO}_2$  gas mixture and adjust pH to 2.5 using 10 N  $\text{H}_2\text{SO}_4$ . 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%  $\text{N}_2$  gas atmosphere. Pressurize inoculated bottles to 1 bar overpressure with sterile 80%  $\text{H}_2$  and 20%  $\text{CO}_2$  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%  $\text{H}_2$  and 20%  $\text{CO}_2$  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%  $\text{H}_2$  and 20%  $\text{CO}_2$  gas mixture.