

**88a. SULFOLOBUS 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	10.00	g
Yeast extract (OXOID)	0.50	g
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, yeast extract and sulfide), adjust pH of the salt solution at room temperature to 4.0 using 1 N  $\text{H}_2\text{SO}_4$  and sparge medium with 100%  $\text{N}_2$  gas to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 1 - 2 hours on each of 3 successive days. Add yeast extract and sulfide from sterile anoxic stock solutions prepared under 100%  $\text{N}_2$  gas. Prior to inoculation check pH and adjust to 4.0, if necessary.

For [DSM 2161](#) and [DSM 2162](#) use 1.00 g/l yeast extract and adjust pH of medium to 5.5.