

88a: SULFOLOBUS MEDIUM (ANAEROBIC)

(NH ₄) ₂ SO ₄	1.30	g
KH ₂ PO ₄	0.28	g
$MgSO_4 \times 7 H_2O$	0.25	g
$CaCl_2 \times 2 H_2O$	0.07	g
$FeCl_3 \times 6 H_2O$	0.02	g
Allen's trace element solution	10.00	ml
Sulfur (powder)	10.00	g
Yeast extract (OXOID)	0.50	g
$Na_2S \times 9 H_2O$	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 $1 \text{ N H}_2\text{SO}_4$ 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% N₂ gas. Prior to inoculation check pH and adjust to 4.0, if necessary.

For <u>DSM 2161</u>, <u>DSM 2162</u>: Use 1.00 g/l yeast extract and adjust pH of medium to 5.5.

Allen's trace element solution (from medium 88)

$MnCl_2 \times 4 H_2O$	180.00	mg
Na ₂ B ₄ O ₇ x 10 H ₂ O	450.00	mg
$ZnSO_4 \times 7 H_2O$	22.00	mg
$CuCl_2 \times 2 H_2O$	5.00	mg
$Na_2MoO_4 \times 2 H_2O$	3.00	mg
$VOSO_4 \times 2 H_2O$	3.00	mg
$CoSO_4 \times 7 H_2O$	1.00	mg
Distilled water	1000.00	ml

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