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** 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 H\(_2\)SO\(_4\) and sparge medium with 100% 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% N\(_2\) gas. Prior to inoculation check pH and adjust to 4.0, if necessary.

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

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

\[\text{MnCl}_2 \times 4 \text{H}_2\text{O}\] 180.00 mg  
\[\text{Na}_2\text{BaO}_7 \times 10 \text{H}_2\text{O}\] 450.00 mg  
\[\text{ZnSO}_4 \times 7 \text{H}_2\text{O}\] 22.00 mg  
\[\text{CuCl}_2 \times 2 \text{H}_2\text{O}\] 5.00 mg  
\[\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}\] 3.00 mg  
\[\text{VOSO}_4 \times 2 \text{H}_2\text{O}\] 3.00 mg  
\[\text{CoSO}_4 \times 7 \text{H}_2\text{O}\] 1.00 mg  
Distilled water 1000.00 ml

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