

897: IGNICOCCUS MEDIUM

MgSO ₄ x 7 H ₂ O 3.50 g MgCl ₂ x 6 H ₂ O 2.75 g KH ₂ PO ₄ 0.50 g (NH ₄) ₂ SO ₄ 0.25 g CaCl ₂ x 2 H ₂ O 0.38 g KCI 0.33 g NaBr 0.05 g H ₃ BO ₃ 15.00 mg SrCl2 x 6 H ₂ O (0.1% w/v) 7.00 ml KI (0.01% w/v) 0.50 ml Sodium resazurin (0.1% w/v) 5.00 g Meat extract 1.00 g Na ₂ S x 9 H ₂ O 0.20 g	NaCl	13.65	g
$\begin{array}{llllllllllllllllllllllllllllllllllll$	$MgSO_4 \times 7 H_2O$	3.50	
$(NH_4)_2SO_4$ 0.25g $(aCl_2 \times 2 H_2O)$ 0.38gKCl0.33gNaBr0.05gH_3BO_315.00mgSrCl2 \times 6 H_2O (0.1% w/v)7.00mlKI (0.01% w/v)0.50mlSodium resazurin (0.1% w/v)0.50mlSulfur (powdered)5.00gMeat extract1.00gNa_2S \times 9 H_2O0.20g	$MgCl_2 \times 6 H_2O$	2.75	-
$\begin{array}{cccc} CaCl_2 \times 2 \ H_2O & 0.38 & g \\ KCl & 0.33 & g \\ NaBr & 0.05 & g \\ H_3BO_3 & 15.00 & mg \\ SrCl2 \times 6 \ H_2O & (0.1\% \ w/v) & 7.00 & ml \\ Kl & (0.01\% \ w/v) & 0.50 & ml \\ Sodium \ resazurin & (0.1\% \ w/v) & 0.50 & ml \\ Sulfur & (powdered) & 5.00 & g \\ Meat \ extract & 1.00 & g \\ Na_2S \times 9 \ H_2O & 0.20 & g \end{array}$	KH ₂ PO ₄	0.50	g
KCl 0.33 gNaBr 0.05 gH_3BO_315.00mgSrCl2 x 6 H_2O (0.1% w/v)7.00mlKI (0.01% w/v)0.50mlSodium resazurin (0.1% w/v)0.50mlSulfur (powdered)5.00gMeat extract1.00gNa_2S x 9 H_2O0.20g	(NH ₄) ₂ SO ₄	0.25	g
NaBr0.05g H_3BO_3 15.00mg $SrCl2 \times 6 H_2O (0.1\% w/v)$ 7.00ml $Kl (0.01\% w/v)$ 0.50mlSodium resazurin (0.1% w/v)0.50mlSulfur (powdered)5.00gMeat extract1.00g $Na_2S \times 9 H_2O$ 0.20g	$CaCl_2 \times 2 H_2O$	0.38	g
$\begin{array}{ccc} H_3BO_3 & 15.00 & mg \\ SrCl2 x 6 H_2O (0.1\% w/v) & 7.00 & ml \\ Kl (0.01\% w/v) & 0.50 & ml \\ Sodium resazurin (0.1\% w/v) & 0.50 & ml \\ Sulfur (powdered) & 5.00 & g \\ Meat extract & 1.00 & g \\ Na_2S x 9 H_2O & 0.20 & g \end{array}$	KCI	0.33	g
SrCl2 x 6 H2O (0.1% w/v)7.00mlKI (0.01% w/v)0.50mlSodium resazurin (0.1% w/v)0.50mlSulfur (powdered)5.00gMeat extract1.00gNa2S x 9 H2O0.20g	NaBr	0.05	g
KI $(0.01\% \text{ w/v})$ 0.50mlSodium resazurin $(0.1\% \text{ w/v})$ 0.50mlSulfur (powdered)5.00gMeat extract1.00gNa2S x 9 H2O0.20g	H ₃ BO ₃	15.00	mg
Sodium resazurin (0.1% w/v)0.50mlSulfur (powdered) 5.00 gMeat extract 1.00 gNa ₂ S x 9 H ₂ O 0.20 g	SrCl2 x 6 H ₂ O (0.1% w/v)	7.00	ml
Sulfur (powdered) 5.00 gMeat extract 1.00 gNa2S x 9 H2O 0.20 g	KI (0.01% w/v)	0.50	ml
Meat extract 1.00 g Na2S x 9 H2O 0.20 g	Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ S x 9 H ₂ O 0.20 g	Sulfur (powdered)	5.00	g
	Meat extract	1.00	g
Distilled water 1000.00 ml	$Na_2S \times 9 H_2O$	0.20	g
	Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur, meat extract and sulfide, adjust pH to 5.0 - 5.5 with 2 N H₂SO₄ and sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials already containing the appropriate amount of sulfur, only to 30% of their volume to allow for a large head space. For sterilization heat cultivation vessels in boiling water for 1 -2 hours on each of 3 successive days. Add meat extract and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere.

2. After inoculation pressurize the vessels with sterile 80% $\rm H_2$ and 20% $\rm CO_2$ gas mixture to 2 bar overpressure.

For <u>DSM 18386</u>: Prepare medium without meat extract.