390. PYROBACULUM MEDIUM

$(NH_4)_2SO_4$	1.30	g
KH_2PO_4	0.28	g
$MgSO_4$ x 7 H_2O	0.25	g
$CaCl_2$ x 2 H_2O	0.07	g
$FeCl_3$ x 6 H_2O	0.02	g
Allen's trace element solution (see medium 88)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na_2S x 9 H_2O	0.50	g
Substrates (see below)		
Distilled water	1000.00	ml

Dissolve ingredients (except sulfide and substrates), adjust pH to 6.0 and sparge medium with 100% N_2 gas for at least 30 min to remove dissolved oxygen. After autoclaving add substrates as listed below from sterile anoxic stock solutions prepared under 100% N_2 gas. Stock solutions of substrates can be autoclaved with the exception of sodium thiosulfate which should be sterilized by filtration. Prior to inoculation add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas and adjust pH of the complete medium to the appropriate value.
Substrates* and additional instructions: *see below!

For DSM 4184:

Trypticase peptone (BD BBL)	0.50	g
Yeast extract	0.20	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g

Adjust pH of complete medium to 6.0.

For DSM 4185:

Trypticase peptone (BD BBL)	0.50	g
Yeast extract	0.20	g
Sulfur	20.00	g

Add peptone and yeast extract from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Sulfure is sterilized by steaming for 3 hours on each of 3 succesive days and added aseptically to the sterile medium while retaining anoxic conditions.
Adjust pH of complete medium to 6.0.

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For DSM 13380:

Yeast extract	1.00	g
$Na_2S_2O_3$ x 5 H_2O	1.00	g

Adjust pH of complete medium to 7.0.

For DSM 13514 and DSM 103086:

Trypticase peptone (BD BBL)	0.50	g
Yeast extract	0.20	g
$Na_2S_2O_3$ x 5 H_2O	2.00	g

Adjust pH of complete medium to 6.8.