

## 390. PYROBACULUM MEDIUM

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.28	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.07	g
FeCl <sub>3</sub> x 6 H <sub>2</sub> O	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 <sub>2</sub> S x 9 H <sub>2</sub> O	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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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 <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	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<sub>2</sub> gas atmosphere. Sulfure is sterilized by steaming for 3 hours on each of 3 successive 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 <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	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 <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	2.00	g

Adjust pH of complete medium to 6.8.