

## 1271: PLEOMORPHOCHAETA MEDIUM

This recipe contains strain-specific modifications for *Spirochaeta psychrophila* DSM 23951 \*

Final pH: 7.3 - 7.5

Final volume: 1003 ml

NH <sub>4</sub> Cl	0.50	g
KH <sub>2</sub> PO <sub>4</sub>	0.10	g
NaCl	25.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.00	g
Yeast extract	1.00	g
<del>D-Glucose</del>	<del>1.70</del>	<del>g</del>
<b>Trace element solution</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Sucrose	2.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, cysteine and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.3 - 7.5, if necessary.

\* Replace glucose with 2.0 g/l sucrose.

### Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### Trace element solution (from medium 732)

Na <sub>2</sub> -EDTA	0.50	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	2.00	g

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ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
AlCl <sub>3</sub>	10.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	1000.00	ml

First dissolve EDTA in distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add remaining compounds.

### Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml