

## 1163a: OCEANOTOGA MEDIUM

NaCl	30.00	g
HEPES (SIGMA)	4.70	g
KCl	0.33	g
MgCl <sub>2</sub> x 2 H <sub>2</sub> O	0.90	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	1.40	g
NH <sub>4</sub> Cl	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
KH <sub>2</sub> PO <sub>4</sub>	0.45	g
Yeast extract	5.00	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
D-Glucose	5.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, maltose and vitamins. Sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic, then add and dissolve cysteine and adjust pH to 7.5 with NaOH. Dispense medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose and vitamins after autoclaving from anoxic stock solutions prepared under 100% N<sub>2</sub> gas and sterilized by filtration.

For DSM 24739: Replace glucose with 5.00 g/l D-xylose and supplement medium after autoclaving with 2.50 g/l Na-thiosulfate added from an anoxic sterile stock solution.

### Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

### **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