

## 1236: SPOROLITUUS MEDIUM

$\text{KH}_2\text{PO}_4$	0.75	g
$\text{K}_2\text{HPO}_4$	1.50	g
NaCl	0.90	g
$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$	0.20	g
$\text{NH}_4\text{Cl}$	0.90	g
<b>Modified Wolin's mineral solution</b>	9.00	ml
Tryptone (BD Bacto)	2.00	g
Yeast extract (OXOID)	2.00	g
<b>FeSO<sub>4</sub> x 7 H<sub>2</sub>O solution (0.1% w/v)</b>	0.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sodium citrate	2.00	g
<b>Wolin's vitamin solution</b>	5.00	ml
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except ferrous sulfate, citrate, vitamins and sulfide), adjust pH to 6.5 and sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add ferrous sulfate and dispense medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add citrate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and adjust pH of complete medium to 7.0, if necessary.

For DSM 25808: Replace citrate with 2.00 g/l Casamino acids (DIFCO).

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

Nitrolotriacetic acid	1.50	g
$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$	3.00	g
$\text{MnSO}_4 \times \text{H}_2\text{O}$	0.50	g
NaCl	1.00	g
$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$	0.10	g
$\text{CoSO}_4 \times 7 \text{ H}_2\text{O}$	0.18	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	0.10	g
$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$	0.18	g
$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$	0.01	g
$\text{AlK(SO}_4)_2 \times 12 \text{ H}_2\text{O}$	0.02	g
$\text{H}_3\text{BO}_3$	0.01	g
$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$	0.01	g
$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$	0.03	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	0.30	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	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** (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B <sub>12</sub>	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

### **FeSO<sub>4</sub> x 7 H<sub>2</sub>O solution (0.1% w/v)** (from medium 119)

FeSO <sub>4</sub> x 7 H <sub>2</sub> O	1.00	g
H <sub>2</sub> SO <sub>4</sub> (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.