

## 483: DEFUVIITALEA MEDIUM

$\text{KH}_2\text{PO}_4$	0.30	g
$\text{K}_2\text{HPO}_4$	0.30	g
$\text{NH}_4\text{Cl}$	1.00	g
$\text{NaCl}$	1.00	g
$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$	1.00	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	0.10	g
$\text{KCl}$	0.10	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x $\text{H}_2\text{O}$	0.50	g
$\text{Na}_2\text{CO}_3$	1.00	g
D-Glucose	4.00	g
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, carbonate, glucose 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. Add cysteine and adjust pH to 6.5, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add glucose and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of complete medium to pH 7.2 -7.4, if necessary.

### 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
$\text{NaCl}$	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.