

## 663: DESULFITOBACTERIUM MEDIUM

KH <sub>2</sub> PO <sub>4</sub>	5.44	g
MgCl <sub>2</sub> x 2 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.03	g
NH <sub>4</sub> Cl	0.50	g
<b>Trace element solution</b>	5.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na-pyruvate	2.50	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	2.00	g
<b>Wolin's vitamin solution (10x)</b>	2.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.40	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	1000.00	ml

Dissolve ingredients (except pyruvate, thiosulfate, vitamins, cysteine and sulfide), then sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate, thiosulfate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Stock solutions of pyruvate and vitamins are sterilized by filtration. Adjust medium pH to 7.5 by adding sterile anoxic 1 N NaOH.

### Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	0.20	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.10	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.17	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnCl <sub>2</sub>	0.10	g
CuCl <sub>2</sub>	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
NaCl	1.00	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

### 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