

**1086: DESULFATIRHABDIUM MEDIUM**

Na <sub>2</sub> HPO <sub>4</sub> x 2 H <sub>2</sub> O	0.53	g
KH <sub>2</sub> PO <sub>4</sub>	0.41	g
NH <sub>4</sub> Cl	0.30	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.11	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
NaCl	0.30	g
Na <sub>2</sub> SO <sub>4</sub>	2.80	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	0.02	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Na-benzoate	0.43	g
<b>Na-crotonate solution (1 M)</b>	20.00	ml
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	980.00	ml

1. Dissolve ingredients (except carbonate, benzoate, crotonate, vitamins and sulfide) and 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. Add benzoate, crotonate, vitamins 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. The crotonate and vitamin solutions should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2.

2. After inoculation pressurize the vessels with sterile 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 0.7 bar overpressure.

**Trace element solution SL-10** (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
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
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	990.00	ml

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First dissolve  $\text{FeCl}_2$  in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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

NaOH	0.50	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$	3.00	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$	4.00	mg
Distilled water	1000.00	ml

### **Na-crotonate solution (1 M)** (from medium 870)

Crotonic acid (ALDRICH 113018)	86.00	g
NaOH (10 N)	100.00	ml
Distilled water	900.00	ml

Dissolve crotonic acid in 800 ml distilled water, add around 100 ml of 10 N NaOH and adjust pH to around 7. Then add water to reach a volume of 1000 ml. Sterilize by filtration under 100%  $\text{N}_2$  gas atmosphere.

### **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