

**1114: THIOFABA MEDIUM**

<b>Solution A</b>	972.00	ml
<b>Solution B</b>	10.00	ml
<b>Solution C</b>	10.00	ml
<b>Solution D</b>	10.00	ml
<b>Solution E</b>	1.00	ml

1. Sparge solution A with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into serum vials (to 20% of volume) and autoclave. Solution B is autoclaved separately under 100% N<sub>2</sub> gas and solution C under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions D and E are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. The pH of the complete medium should be 6.5.

2. After inoculation cultivation vials are pressurized with an amount of sterile air that is equivalent to 30 vol% of the cultivation vial.

**Solution A**

MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.75	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
NH <sub>4</sub> Cl	0.54	g
<b>Modified Wolin's mineral solution</b>	2.00	ml
Distilled water	970.00	ml

**Solution B**

KH <sub>2</sub> PO <sub>4</sub>	1.19	g
K <sub>2</sub> HPO <sub>4</sub>	0.21	g
Distilled water	10.00	ml

**Solution C**

Na <sub>2</sub> CO <sub>3</sub>	0.50	g
Distilled water	10.00	ml

**Solution D**

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	1.25	g
Distilled water	10.00	ml

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### Solution E

<b>Wolin's vitamin solution (10x)</b>	1.00	ml
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### Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic 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