

## 1267c: FERRIGENEUM MEDIUM

Final pH: 6.5

Final volume: 961 ml

<b>Solution A</b>	880.00	ml
<b>Solution B</b>	1.00	ml
<b>Solution C</b>	80.00	ml

1. **Preparation of the bottom layer:** Purge solution A (except NaHCO<sub>3</sub> and agarose) with 100% N<sub>2</sub> gas to make it anoxic and add 0.80 ml of the solution per tube under N<sub>2</sub> gas atmosphere to Hungate-type tubes (16 ml total volume) already containing 15 mg agarose type 1, low EEO. Then 0.80 ml of solution C is added to each tube under an N<sub>2</sub> gas atmosphere. Thereafter, tubes are sealed and autoclaved. The bottom layer solidifies in approx. 30 min.

2. **Preparation of the top layer:** Purge solution A with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to make it anoxic, then add bicarbonate and low-melting agarose, adjust the pH to 6.5 and autoclave under same gas atmosphere. Allow the sterile solution to cool to 40°C and add aseptically 1.00 ml/l of anoxic sterile solution B. Then pipette 8.0 ml aseptically over the bottom layer of each tube under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere, reseal the tubes and allow the medium equilibrate for at least four hours, but not longer than 24 hours.

3. **Inoculation:** Open the tube under air atmosphere and inoculate the semisolid top layer with a pipette that is inserted just above the FeS layer; the pipette tip is drawn upward as the inoculum is dispensed.

### Solution A

NH <sub>4</sub> Cl	1.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.20	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
K <sub>2</sub> HPO <sub>4</sub>	0.05	g
<b>Wolfe's mineral elixir</b>	1.00	ml
<b>Arsenate-Vanadium solution</b>	1.00	ml
NaHCO <sub>3</sub> (for top layer)	0.50	g
Agarose (for top layer, low melt)	1.50	g
Distilled water	1000.00	ml

### Solution B (from medium 1267b)

<b>Wolin's vitamin solution (10x)</b>	1.00	ml
---------------------------------------	------	----

## 1267c: FERRIGENEUM MEDIUM

### Solution C (from medium 1267b)

<b>Ferrous sulfide sludge</b>	100.00	ml
-------------------------------	--------	----

### Wolfe's mineral elixir (from medium 792)

MgSO <sub>4</sub> × 7 H <sub>2</sub> O	30.00	g
MnSO <sub>4</sub> × H <sub>2</sub> O	5.00	g
NaCl	10.00	g
FeSO <sub>4</sub> × 7 H <sub>2</sub> O	1.00	g
CoCl <sub>2</sub> × 6 H <sub>2</sub> O	1.80	g
CaCl <sub>2</sub> × 2 H <sub>2</sub> O	1.00	g
ZnSO <sub>4</sub> × 7 H <sub>2</sub> O	1.80	g
CuSO <sub>4</sub> × 5 H <sub>2</sub> O	0.10	g
AlK(SO <sub>4</sub> ) <sub>2</sub> × 12 H <sub>2</sub> O	0.18	g
H <sub>3</sub> BO <sub>3</sub>	0.10	g
Na <sub>2</sub> MoO <sub>4</sub> × 2 H <sub>2</sub> O	0.10	g
(NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> ) <sub>2</sub> × 6 H <sub>2</sub> O	2.80	g
Na <sub>2</sub> WO <sub>4</sub> × 2 H <sub>2</sub> O	0.10	g
Na <sub>2</sub> SeO <sub>4</sub>	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H<sub>2</sub>SO<sub>4</sub>, then add and dissolve the salts.

### Arsenate-Vanadium solution (from medium 1823)

Na <sub>2</sub> HAsO <sub>4</sub> × 7 H <sub>2</sub> O	156.00	mg
VO <sub>2</sub> SO <sub>4</sub> × H <sub>2</sub> O (0.1% w/v)	1.00	ml
Distilled water	1000.00	ml

### Ferrous sulfide sludge (from medium 1267)

FeSO <sub>4</sub> × 7 H <sub>2</sub> O	15.40	g
Na <sub>2</sub> S × 9 H <sub>2</sub> O	12.30	g
Distilled water	100.00	ml

Heat distilled water to 50°C in a 250 ml beaker with a stir bar present. While rapidly stirring the water, add the ferrous sulfate followed immediately by the sodium sulfide. The formed black FeS sludge is decanted into a glass bottle that can be stoppered. The FeS is allowed to settle for several hours and then the overlying water is decanted and replaced. This procedure is repeated at least five times to wash the FeS. After washing, the pH of the FeS solution should be close to neutrality. The FeS suspension can be kept in closed bottles or tubes under a nitrogen atmosphere for at least three months.

### Wolin's vitamin solution (10x) (from medium 120)



## 1267c: FERRIGENEUM MEDIUM

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