1210b: PYRODICTIUM DELANEYI MEDIUM

NaCl	18.00	g
$MgCl_2$ x 6 H_2O	4.00	g
KCl	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
$(NH_4)_2SO_4$	0.50	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1 % w/v)	0.50	ml
Na_2CO_3	1.00	g
KH_2PO_4	0.33	g
$FeCl_2$ x 4 H_2O	0.52	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.10	g
Amorphous $Fe(OH)_3$	100.00	ml
Distilled water	900.00	ml

Dissolve ingredients except carbonate, hydrogenphosphate, ferrous chloride, yeast extract, vitamins, cysteine and ferric iron hydroxide sludge. Suspend pellet of ferric iron hydroxide in medium and sparge with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Thereafter, dispense suspension under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave for 30 min. Add hydrogenphosphate, ferrous chloride (dissolved in 0.1 N HCl), yeast extract, cysteine and vitamins from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins and ferrous chloride should be sterilized by filtration. The pH of the complete medium should be at 5.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ 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
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Amorphous $Fe(OH)_3$ (from medium 1210)

$FeCl_3$ x 6 H_2O (60.00 g/l)	320.00	ml
NaOH (10% w/v), adjust if required	80.00	ml

Slowly titrate 320 ml of a $FeCl_3$ x 6 H_2O stock solution (60.00 g/l) with 10% (w/v) NaOH to pH 8.0-8.5 under agitation (use magnetic stirrer). Total amount of added NaOH approx. 80 - 100 ml. The precipitated $Fe(OH)_3$ should be stored at room temperature overnight with surface covered with water. Thereafter, centrifuge at 2000 rpm for 5 min and discard the supernatant. Wash several times with distilled water. Resuspend the pellet in medium as described above. For storage autoclave under 100% N_2 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_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml