

**1689. NITROSPIRA INOPINATA MEDIUM**

Medium stock solution, 20fold

KH <sub>2</sub> PO <sub>4</sub>	1.000	g
KCl	1.500	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	1.000	g
NaCl	11.680	g
Distilled (MilliQ) water	1000.00	ml

Dilute stock solution with MilliQ water (1+19 v/v), add 4 g CaCO<sub>3</sub> per litre and autoclave. After autoclaving add sterile solutions:

1 ml Trace element solution,

1 ml selenite-tungstate solution (see below),

1 ml borate-copper solution (see below)

**10 ml ammonium chloride solution** (0.8 g in 100 ml MilliQ water, 150 mM)

Complete medium can be stored at 4°C.

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

HCl (25%; 7.7 M)	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

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

*Selenite-tungstate solution* (see medium 385)

NaOH	0.5	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.0	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.0	mg
Distilled water	1000.0	ml

*Borate-copper solution*

H <sub>3</sub> BO <sub>3</sub>	54	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	18	mg
Distilled water	1000.0	ml

Ctd.

Incubate at 42°C without shaking. Activity and growth can be followed by measuring the depletion of ammonium or the production of nitrite and/or nitrate. Higher ammonium concentrations are inhibitive. For that reason our recommendation is follow nitrite content. As soon as nitrite has been produced and is depleting again (to form nitrate) another dose of ammonium can be added. Incubation time may be up to several weeks. We checked the nitrite content (absence/presence) every 5-7 days and added ammonium chloride about every 2-3 weeks. Don't expose cultures to intense light.