1690: NITROSPIRA INOPINATA MEDIUM

KH_2PO_4	1.00	g
KCl	1.50	g
$MgSO_4$ x 7 H_2O	1.00	g
NaCl	11.68	g
Distilled water (MilliQ)	1000.00	ml
$CaCO_3$	4.00	g

1. Dilute stock solution with MilliQ water (1+19 v/v), add 4 g $CaCO_3$ per litre and autoclave. After autoclaving add sterile solutions:

Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Borate-copper solution	1.00	ml
Ammonium chloride solution	10.00	ml

2. Complete medium can be stored at 4°C.

3. Check pH 7.6-8.0

For DSM 105286: Do not shake cultures. Growth takes place without visible turbidity and may be best detected by measuring ammonium and/or nitrite or nitrate concentrations in the culture broth

Borate-copper solution

H_3BO_3	54.00	mg
$CuCl_2$ x 2 H_2O	18.00	mg
Distilled water	1000.00	ml

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.

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

Ammonium chloride solution

Ammonium chloride	0.80	g
Distilled water	100.00	ml