380: MAGNETOSPIRILLUM MEDIUM

KH_2PO_4	0.68	g
$NaNO_3$	0.12	g
L(+)-Tartaric acid	0.37	g
Succinic acid	0.37	g
Na-acetate	0.05	g
Yeast extract	0.10	g
Modified Wolin's mineral solution	5.00	ml
Fe(III) quinate solution 0.01 M	2.00	ml
Agar (BD Bacto), for semi-solid medium (optional)	1.30	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na-thioglycolate	0.05	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except thioglycolate and vitamins) in the order given and adjust pH to 6.75 with NaOH.

2. Preparation of liquid medium: Sparge medium with 100% N_2 gas for 30 -45 min and dispense under the same gas atmosphere into anoxic Hungate-type tubes to 50% of their volume. Seal vials with screw caps and gas tight butyl rubber closures. Autoclave at 121°C for 15 min. Before inoculation add thioglycolate and vitamins from stock solutions prepared under 100% N_2 gas and filter-sterilized. Then add sterile air (with hypodermic syringe through the rubber closure) to a concentration of ca. 1% (v/v) O_2 in the vial (e.g., add 1 ml air to a Hungate-type tube of 16 ml total volume).

3. Preparation of semi solid medium: Supplement medium with agar, bring medium to the boil and cool under 100% N_2 gas atmosphere. Dispense under same gas atmosphere aliquots of 10 ml semi-solid medium into Hungate-type tubes. Prior to inoculation add thioglycolate from a 0.5% (w/v) stock solution, freshly prepared under 100% N_2 gas and filter-sterilized. Then add sterile air (with hypodermic syringe through the rubber closure) to a concentration of ca. 1% (v/v) in the vial.

4. Note: Prior to inoculation media should be slightly pink in color. Strongly reduced conditions will not support growth of microaerophilic Magnetospirillum species. Use as inoculum 10% (v/v). Incubate tubes with medium without agitation in an inclined position. During growth O_2 will be consumed and the pH will increase. For cultivation of magnetic cells we recommend preparation of liquid medium, while semi-solid medium is more suitable for demonstration of microaerophilic band formation and storage.

For DSM 6361: Increase the amount of added O_2 to a concentration of 5% (v/v) in the vial (e.g., add 4 ml sterile air to a Hungate-type tube of 16 ml total volume).

For DSM 29233: Supplement medium with 0.25 g/l sodium thiosulfate.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	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.

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml

Fe(III) quinate solution 0.01 M (from medium 380)

$FeCl_3$ x 6 H_2O	4.50	g
Quinic acid	1.90	g
Distilled water	1000.00	ml

Sterilize by filtration under 100% N_2 gas atmosphere.