

## 1024: NITRATIRUPTOR AND NITRATIFRACTOR MEDIUM

This recipe contains strain-specific modifications for *Nitratiruptor tergarcius* DSM 16512 \*

NaNO <sub>3</sub>	1.00	g
NaHCO <sub>3</sub>	1.00	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	1.00	g
Sulfur powder	3.00	g
<b>Wolin's vitamin solution</b>	10.00	ml
<b>Modified Wolin's mineral solution II</b>	10.00	ml
<b>Synthetic seawater</b>	1000.00	ml

1. Prepare the medium under an atmosphere of H<sub>2</sub>/CO<sub>2</sub> (80:20) without adding the vitamins and NaHCO<sub>3</sub> in serum bottles and seal the serum tubes with butyl rubber stoppers. Steam medium for 3 hours on each of 3 successive days. To the sterile medium add, from filter sterilised stock solutions, the NaHCO<sub>3</sub> and vitamin solution.

2. Increase the 80% H<sub>2</sub> + 20% CO<sub>2</sub> gas phase pressure to 300 kPa. The final pH is 7.0.

\* 80% H<sub>2</sub> plus 20% CO<sub>2</sub> at 350 kPa

### Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B <sub>12</sub>	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml

### Synthetic seawater (from medium 997)

NaCl	30.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
NH <sub>4</sub> Cl	0.25	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.18	g
KCl	0.33	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.50	mg
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.50	mg

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$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6 \text{H}_2\text{O}$	0.01	g
<b>Trace mineral solution</b>	10.00	ml

### **Trace mineral solution** (from medium 997)

$\text{C}_6\text{H}_9\text{NO}_6$	1.500	g
$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$	3.000	g
$\text{MnSO}_4 \times 2 \text{H}_2\text{O}$	0.500	g
NaCl	1.000	g
$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$	0.100	g
$\text{CoSO}_4 \times 7 \text{H}_2\text{O}$	0.180	g
$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$	0.100	g
$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$	0.180	g
$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$	0.010	g
$\text{AlK}(\text{SO}_4)_2 \times 12 \text{H}_2\text{O}$	0.020	g
$\text{H}_3\text{BO}_3$	0.010	g
$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$	0.010	g
$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$	0.025	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$	0.300	mg
Distilled water	1000.000	ml

### **Modified Wolin's mineral solution II** (from medium 700)

Nitrilotriacetic acid	1.500	g
$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$	3.000	g
$\text{MnSO}_4 \times \text{H}_2\text{O}$	0.500	g
NaCl	1.000	g
$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$	0.100	g
$\text{CoSO}_4 \times 7 \text{H}_2\text{O}$	0.180	g
$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$	0.100	g
$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$	0.180	g
$\text{CuSO}_4 \times 5 \text{H}_2\text{O}$	0.010	g
$\text{AlK}(\text{SO}_4)_2 \times 12 \text{H}_2\text{O}$	0.020	g
$\text{H}_3\text{BO}_3$	0.010	g
$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$	0.010	g
$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$	0.025	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$	0.300	mg
Distilled water	1000.000	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.