

1024: NITRATIRUPTOR AND NITRATIFRACTOR MEDIUM

This recipe contains strain-specific modifications for *Rhizobiales* (not further classified) DSM 23458 *

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

1. Prepare the medium under an atmosphere of H₂/CO₂ (80:20) without adding the vitamins and NaHCO₃ 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 NaHCO₃ and vitamin solution.

2. Increase the 80% H₂ + 20% CO₂ gas phase pressure to 300 kPa. The final pH is 7.0.

* PH 6.8

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 ₁₂ | 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 ₂ HPO ₄ | 0.14 | g |
| CaCl ₂ x 2 H ₂ O | 0.14 | g |
| NH ₄ Cl | 0.25 | g |
| MgSO ₄ x 7 H ₂ O | 3.40 | g |
| MgCl ₂ x 6 H ₂ O | 4.18 | g |
| KCl | 0.33 | g |
| NiCl ₂ x 6 H ₂ O | 0.50 | mg |

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| | | |
|---|-------|----|
| $\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$ | 0.50 | mg |
| $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6 \text{H}_2\text{O}$ | 0.01 | g |
| Trace mineral solution | 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 |
| H_3BO_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 |
| H_3BO_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.