

1104: DETHIOBACTER MEDIUM

This recipe contains strain-specific modifications for *Desulfurivibrio alkaliphilus* DSM 19089 *

Final pH: 9.5

Final volume: 1004 ml

| | | |
|--------------------------------------------------------------------|-----------------|---------------|
| NaCl | 6.00 | g |
| K ₂ HPO ₄ | 0.50 | g |
| NaHCO ₃ | 8.00 | g |
| Na ₂ CO ₃ | 22.00 | g |
| Sulfur (powdered) | 1.50 | g |
| NH ₄ Cl | 0.20 | g |
| MgCl ₂ x 6 H ₂ O | 0.20 | g |
| Trace elements solution (Pfennig & Lippert,1966) | 1.00 | ml |
| Selenite-tungstate solution | 1.00 | ml |
| Yeast extract | 0.05 | g |
| Ethanol absolute | 0.60 | ml |
| Na-acetate | 0.40 | g |
| Na ₂ S ₂ O ₃ x 5 H ₂ O | 2.50 | g |
| Wolin's vitamin solution (10x) | 1.00 | ml |
| Na ₂ S x 9 H ₂ O | 0.24 | g |
| Sodium butyrate | 1.10 | g |
| Distilled water | 1000.00 | ml |

1. Dissolve sodium chloride and hydrogenphosphate, then sparge solution with 100% N₂ gas for 30 -45 min to make it anoxic. Add and dissolve carbonates, then dispense under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Sterilize medium by heating cultivation vessels in a water bath to 90 - 100°C for 1- 2 hours on each of 3 successive days. After sterilization add ammonium chloride, magnesium chloride, trace elements, yeast extract, ethanol, acetate, thiosulfate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The stock solutions of thiosulfate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 9.5.

2. After inoculation add sterile 100% H₂ gas to 0.5 bar overpressure.

* Replace ethanol with 1.10 g/l sodium butyrate.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

| | | |
|----------------------------------------|------|---|
| EDTA | 5.00 | g |
| FeSO ₄ x 7 H ₂ O | 2.20 | g |
| ZnSO ₄ x 7 H ₂ O | 0.10 | g |
| MnCl ₂ x 4 H ₂ O | 0.03 | g |

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| | | |
|-------------------------------------------------------|---------|----|
| H ₃ BO ₃ | 0.03 | g |
| CoCl ₂ x 6 H ₂ O | 0.20 | g |
| CuCl ₂ x 2 H ₂ O | 0.03 | g |
| NiCl ₂ x 6 H ₂ O | 0.03 | g |
| Na ₂ MoO ₄ x 2 H ₂ O | 0.03 | g |
| Distilled water | 1000.00 | ml |

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

| | | |
|-------------------------------------------------------|---------|----|
| NaOH | 0.50 | g |
| Na ₂ SeO ₃ x 5 H ₂ O | 3.00 | mg |
| Na ₂ WO ₄ x 2 H ₂ O | 4.00 | mg |
| Distilled water | 1000.00 | ml |

Wolin's vitamin solution (10x) (from medium 120)

| | | |
|----------------------------|---------|----|
| Biotin | 20.00 | mg |
| Folic acid | 20.00 | mg |
| Pyridoxine hydrochloride | 100.00 | mg |
| Thiamine HCl | 50.00 | mg |
| Riboflavin | 50.00 | mg |
| Nicotinic acid | 50.00 | mg |
| Calcium D-(+)-pantothenate | 50.00 | mg |
| Vitamin B ₁₂ | 1.00 | mg |
| p-Aminobenzoic acid | 50.00 | mg |
| (DL)-alpha-Lipoic acid | 50.00 | mg |
| Distilled water | 1000.00 | ml |