391. METHANOCOCCUS VOLTAE MEDIUM

Mineral salt solution 2xW (see below) 500.00 ml
Trace element solution (see medium 141) 10.00 ml
LIP-solution (see below) 50.00 ml
Na-acetate 1.00 g
Na-resazurin solution (0.1% w/v) 0.50 ml
TYC-solution (see next page) 50.00 ml
Na₂CO₃ 1.50 g
L-Cysteine-HCl x H₂O 0.50 g
Na₂S x 9 H₂O 0.50 g
Distilled water 400.00 ml

Dissolve ingredients except TYC solution, carbonate, cystein and sulfide. Sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Before use add TYC solution, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 7.2, if necessary.

**Mineral solution 2xW:**
- NaCl 40.00 g
- MgCl₂ x 6 H₂O 5.60 g
- MgSO₄ x 7 H₂O 0.70 g
- KCl 0.68 g
- NH₄Cl 0.50 g
- K₂HPO₄ 0.28 g
- CaCl₂ x 2 H₂O 0.28 g
- Distilled water 1000.00 ml

**LIP-solution:**
- L-Leucine 5.00 g
- L-Isoleucine 10.00 g
- Pantothenate 0.10 g
- Distilled water 1000.00 ml

May be stored frozen without sterilization.

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**TYC-solution:**
- Casamino acids (BD Bacto) 100.00 g
- Yeast extract (OXOID) 50.00 g
- L-Tryptophan 1.00 g
- Distilled water 1000.00 ml

Prepare and autoclave under 100% N₂ gas.

For strain DSM 4254 supplement medium with 80.00 mg/l L-histidine added from a sterile anoxic stock solution sterilized by filtration.

For strain DSM 4310 supplement medium with 32.00 mg/l of hypoxanthine and 1.00 ml/l of a vitamins solution (see medium 503) added from anoxic stock solutions sterilized by filtration.