

120a: METHANOSARCINA BARKERI MEDIUM

This recipe contains strain-specific modifications for *Methanosarcina lacustris* DSM 10334 *

Final pH: * 6.8 - 7.0

Final volume: 1024 ml

| | | |
|--|-------------|-----------|
| K ₂ HPO ₄ | 0.35 | g |
| KH ₂ PO ₄ | 0.23 | g |
| NH ₄ Cl | 0.50 | g |
| MgSO ₄ x 7 H ₂ O | 0.50 | g |
| CaCl ₂ x 2 H ₂ O | 0.25 | g |
| NaCl | 2.25 | g |
| FeSO₄ x 7 H₂O solution (0.1% w/v) | 2.00 | ml |
| Trace element solution SL-10 | 1.00 | ml |
| Yeast extract (OXOID) | 2.00 | g |
| Casitone (BD BBL) | 2.00 | g |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| NaHCO₃ | 2.00 | g |
| Methanol (50% v/v) | 5.00 | ml |
| Wolin's vitamin solution (10x) | 1.00 | ml |
| L-Cysteine HCl x H ₂ O | 0.30 | g |
| Na ₂ S x 9 H ₂ O | 0.30 | g |
| Distilled water | 1000.00 | ml |

Dissolve ingredients (except bicarbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Then add and dissolve bicarbonate, adjust pH to 6.5 and dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N₂ gas atmosphere as concentrated solutions in tightly closed tubes. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Appropriate volumes of the solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.5 - 6.8, if necessary.

* Increase amount of NaHCO₃ to 2.00 g/l to achieve a pH of 6.8 - 7.0 and add only 5.00 ml/l methanol.

FeSO₄ x 7 H₂O solution (0.1% w/v) (from medium 119)

| | | |
|--|---------|----|
| FeSO ₄ x 7 H ₂ O | 1.00 | g |
| H ₂ SO ₄ (0.1 N) | 1000.00 | ml |

The ferrous sulfate solution is not stable and should be freshly prepared.

120a: METHANOSARCINA BARKERI MEDIUM

Trace element solution SL-10 (from medium 320)

| | | |
|---|--------|----|
| HCl (25%) | 10.00 | ml |
| FeCl ₂ x 4 H ₂ O | 1.50 | g |
| ZnCl ₂ | 70.00 | mg |
| MnCl ₂ x 4 H ₂ O | 100.00 | mg |
| H ₃ BO ₃ | 6.00 | mg |
| CoCl ₂ x 6 H ₂ O | 190.00 | mg |
| CuCl ₂ x 2 H ₂ O | 2.00 | mg |
| NiCl ₂ x 6 H ₂ O | 24.00 | mg |
| Na ₂ MoO ₄ x 2 H ₂ O | 36.00 | mg |
| Distilled water | 990.00 | ml |

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 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 |