

## 141: METHANOGENIUM MEDIUM (H<sub>2</sub>/CO<sub>2</sub>)

This recipe contains strain-specific modifications for *Methanogenium marinum* DSM 15558 \*

Final pH: \* 6.5

Final volume: 1013 ml

|   |             |          |
|---|-------------|----------|
| KCl   | 0.34        | g        |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O  | 4.00        | g        |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O  | 3.45        | g        |
| NH <sub>4</sub> Cl  | 0.25        | g        |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O  | 0.14        | g        |
| K <sub>2</sub> HPO <sub>4</sub>   | 0.14        | g        |
| NaCl  | 18.00       | g        |
| <b>Modified Wolin's mineral solution</b>  | 10.00       | ml       |
| Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v) | 2.00        | ml       |
| Na-acetate  | 1.00        | g        |
| Yeast extract (OXOID)   | 2.00        | g        |
| Trypticase peptone (BD BBL)   | 2.00        | g        |
| Sodium resazurin (0.1% w/v)   | 0.50        | ml       |
| <b>Wolin's vitamin solution</b>   | 10.00       | ml       |
| NaHCO <sub>3</sub>  | 5.00        | g        |
| L-Cysteine HCl x H <sub>2</sub> O   | 0.50        | g        |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O  | 0.50        | g        |
| <b>2-Mercaptoethanesulfonic acid</b>  | <b>0.50</b> | <b>g</b> |
| Distilled water   | 1000.00     | ml       |

- Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.
- Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 6.5, then dispense medium under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N<sub>2</sub> gas. Vitamins are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.
- For incubation use sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture at two atmospheres of pressure.

\* Supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under 100% N<sub>2</sub> gas. Adjust pH to 6.5 and use only one atmosphere overpressure of sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

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### Modified Wolin's mineral solution (from medium 141)

|  |         |    |
|--|---------|----|
| Nitrilotriacetic acid                                    | 1.50    | g  |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O                   | 3.00    | g  |
| MnSO <sub>4</sub> x H <sub>2</sub> O                     | 0.50    | g  |
| NaCl   | 1.00    | g  |
| FeSO <sub>4</sub> x 7 H <sub>2</sub> O                   | 0.10    | g  |
| CoSO <sub>4</sub> x 7 H <sub>2</sub> O                   | 0.18    | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O                   | 0.10    | g  |
| ZnSO <sub>4</sub> x 7 H <sub>2</sub> O                   | 0.18    | g  |
| CuSO <sub>4</sub> x 5 H <sub>2</sub> O                   | 0.01    | g  |
| AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O | 0.02    | g  |
| H <sub>3</sub> BO <sub>3</sub>                           | 0.01    | g  |
| Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O    | 0.01    | g  |
| NiCl <sub>2</sub> x 6 H <sub>2</sub> O                   | 0.03    | g  |
| Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O    | 0.30    | mg |
| Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O     | 0.40    | mg |
| Distilled water  | 1000.00 | ml |

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

### 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 |