

## 968b: MLMS-1 MEDIUM (ARSENATE)

This recipe contains strain-specific modifications for DSM 17814 \*

Final pH: 9.8

Final volume: 1003 ml

|                                                        |         |    |
|--------------------------------------------------------|---------|----|
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>        | 0.10    | g  |
| MgSO <sub>4</sub>                                      | 25.00   | mg |
| NaCl                                                   | 60.00   | g  |
| K <sub>2</sub> HPO <sub>4</sub>                        | 0.15    | g  |
| KH <sub>2</sub> PO <sub>4</sub>                        | 0.08    | g  |
| <b>Trace element solution SL-10</b>                    | 1.00    | ml |
| <b>Selenite-tungstate solution</b>                     | 1.00    | ml |
| Sodium resazurin (0.1% w/v)                            | 0.50    | ml |
| Na <sub>2</sub> CO <sub>3</sub>                        | 10.60   | g  |
| NaHCO <sub>3</sub>                                     | 4.20    | g  |
| Na <sub>2</sub> HAsO <sub>4</sub> x 7 H <sub>2</sub> O | 3.10    | g  |
| <b>Wolin's vitamin solution (10x)</b>                  | 1.00    | ml |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O                 | 0.82    | g  |
| Distilled water                                        | 1000.00 | ml |

Dissolve ingredients (except carbonates, arsenate, vitamins and sulfide), sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add solid carbonate and hydrogencarbonate, adjust pH to 9.8, dispense medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation, add arsenate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 9.8, if necessary.

\*

### Trace element solution SL-10 (from medium 320)

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

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally

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make up to 1000.00 ml.

### Selenite-tungstate solution (from medium 385)

|                                                       |         |    |
|-------------------------------------------------------|---------|----|
| NaOH                                                  | 0.50    | g  |
| Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O | 3.00    | mg |
| Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> 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 <sub>12</sub>    | 1.00    | mg |
| p-Aminobenzoic acid        | 50.00   | mg |
| (DL)-alpha-Lipoic acid     | 50.00   | mg |
| Distilled water            | 1000.00 | ml |