

## 465: MINERAL MEDIUM PH 7.25

This recipe contains strain-specific modifications for *Herbaspirillum* sp. DSM 12579 \*

Final pH: 7.2

Final volume: 1000 ml

|                                                        |         |    |
|--------------------------------------------------------|---------|----|
| Na <sub>2</sub> HPO <sub>4</sub> x 2 H <sub>2</sub> O  | 3.50    | g  |
| KH <sub>2</sub> PO <sub>4</sub>                        | 1.00    | g  |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>        | 0.50    | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O                 | 0.10    | g  |
| Ca(NO <sub>3</sub> ) <sub>2</sub> x 4 H <sub>2</sub> O | 0.05    | g  |
| <b>Trace element solution SL-4</b>                     | 1.00    | ml |
| Dimethylmalonate                                       | 5.00    | mM |
| Sodium nitrate                                         | 10.00   | mM |
| Distilled water                                        | 1000.00 | ml |

1. pH 7.25
2. Rehydrate and cultivate lyophilized cells in the complex medium recommended for the specific strain (e.g. medium 1, 220, 535 or 830). After this reactivation, cultivate on mineral medium with the appropriate carbon source.

\* With 5 mM sodium dimethylmalonate and 10 mM sodium nitrate; anaerobic

### Main sol. 1 (from medium 1)

|                        |         |    |
|------------------------|---------|----|
| Peptone                | 5.00    | g  |
| Meat extract           | 3.00    | g  |
| Agar, for solid medium | 15.00   | g  |
| Distilled water        | 1000.00 | ml |

1. Adjust pH to 7.0.
2. For Bacillus strains the addition of 10.0 mg MnSO<sub>4</sub> x H<sub>2</sub>O is recommended for sporulation.

### Main sol. 220 (from medium 220)

|                 |         |    |
|-----------------|---------|----|
| Casein peptone  | 15.00   | g  |
| Soy peptone     | 5.00    | g  |
| NaCl            | 5.00    | g  |
| Agar            | 15.00   | g  |
| Distilled water | 1000.00 | ml |

Adjust pH to 7.3. Medium is identical with Tryptone Soya Agar (Oxoid CM 131).

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### Main sol. 535 (from medium 535)

|                                                              |         |    |
|--------------------------------------------------------------|---------|----|
| Trypticase soy broth (BBL 11768, Oxoid CM129 or Merck 30469) | 30.00   | g  |
| Agar                                                         | 20.00   | g  |
| Distilled water                                              | 1000.00 | ml |

1. pH 7.3
2. Autoclave at 121°C for 15 min.

### Main sol. 830 (from medium 830)

|                                        |         |    |
|----------------------------------------|---------|----|
| Yeast extract                          | 0.50    | g  |
| Proteose peptone (Difco no. 3)         | 0.50    | g  |
| Casamino acids                         | 0.50    | g  |
| Glucose                                | 0.50    | g  |
| Starch (soluble)                       | 0.50    | g  |
| Na-pyruvate                            | 0.30    | g  |
| K <sub>2</sub> HPO <sub>4</sub>        | 0.30    | g  |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O | 0.05    | g  |
| Agar                                   | 15.00   | g  |
| Distilled water                        | 1000.00 | ml |

Final pH 7.2; adjust with crystalline K<sub>2</sub>HPO<sub>4</sub> or KH<sub>2</sub>PO<sub>4</sub> before adding agar. Add agar, heat medium to boiling to dissolve agar, and autoclave for 15 min at 121°C.

### Trace element solution SL-4 (from medium 14)

|                                                       |         |    |
|-------------------------------------------------------|---------|----|
| Na <sub>2</sub> -EDTA                                 | 0.50    | g  |
| FeSO <sub>4</sub> x 7 H <sub>2</sub> O                | 0.20    | g  |
| ZnSO <sub>4</sub> x 7 H <sub>2</sub> O                | 0.10    | g  |
| MnCl <sub>2</sub> x 4 H <sub>2</sub> O                | 0.03    | g  |
| H <sub>3</sub> BO <sub>3</sub>                        | 0.30    | g  |
| CoCl <sub>2</sub> x 6 H <sub>2</sub> O                | 0.20    | g  |
| CuCl <sub>2</sub> x 2 H <sub>2</sub> O                | 0.01    | g  |
| NiCl <sub>2</sub> x 6 H <sub>2</sub> O                | 0.02    | g  |
| Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O | 0.03    | g  |
| Distilled water                                       | 1000.00 | ml |

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.