

## 879: CLOSTRIDIUM LJUNGDAHLII MEDIUM

This recipe contains strain-specific modifications for *Caldanaerobius polysaccharolyticus* DSM 13641 \*

Final pH: \* 6.9

Final volume: 1011 ml

|  |                 |              |
|--|-----------------|--------------|
| NH <sub>4</sub> Cl                       | 1.00            | g            |
| KCl                                      | 0.10            | g            |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O   | 0.20            | g            |
| NaCl                                     | 0.80            | g            |
| KH <sub>2</sub> PO <sub>4</sub>          | 0.10            | g            |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O   | 0.02            | g            |
| <b>Modified Wolin's mineral solution</b> | 10.00           | ml           |
| Sodium resazurin (0.1% w/v)              | 0.50            | ml           |
| Yeast extract                            | 1.00            | g            |
| NaHCO <sub>3</sub>                       | 2.50            | g            |
| <del>D-Fructose</del>                    | <del>5.00</del> | <del>g</del> |
| <b>Wolin's vitamin solution (10x)</b>    | 1.00            | ml           |
| L-Cysteine HCl x H <sub>2</sub> O        | 0.30            | g            |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O   | 0.30            | g            |
| Sucrose                                  | 5.00            | g            |
| Distilled water                          | 1000.00         | ml           |

Dissolve ingredients (except bicarbonate, yeast extract, fructose, vitamins, and reducing agents), sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic and adjust pH to 5.0. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave at 121°C for 15 min. Add yeast extract, fructose, vitamins (sterilized by filtration), cysteine, and sulfide from sterile stock solutions prepared under a 100% N<sub>2</sub> gas atmosphere and bicarbonate from a sterile stock solution prepared under an 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust the pH of the complete medium to 5.9!

\* Replace fructose with 5.00 g/l sucrose added to the autoclaved medium from a sterile anoxic stock solution and increase amount of NaHCO<sub>3</sub> to 2.50 g/l. Adjust pH of complete medium to 6.9!

### 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 |

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|  |         |    |
|--|---------|----|
| 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 (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 |