

## 915: BLAUTIA MEDIUM

|   |        |    |
|---|--------|----|
| K <sub>2</sub> HPO <sub>4</sub>                 | 0.30   | g  |
| KH <sub>2</sub> PO <sub>4</sub>                 | 0.30   | g  |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 0.30   | g  |
| NH <sub>4</sub> Cl                              | 1.00   | g  |
| NaCl  | 0.61   | g  |
| MgSO <sub>4</sub> x 7 H <sub>2</sub> O          | 0.12   | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O          | 0.08   | g  |
| <b>Modified Wolin's mineral solution</b>        | 10.00  | ml |
| <b>Clarified rumen fluid</b>                    | 100.00 | ml |
| Yeast extract                                   | 2.00   | g  |
| Sodium resazurin (0.1% w/v)                     | 0.50   | ml |
| NaHCO <sub>3</sub>                              | 7.50   | g  |
| D-Glucose                                       | 5.00   | g  |
| <b>Wolin's vitamin solution (10x)</b>           | 2.00   | ml |
| L-Cysteine HCl x H <sub>2</sub> O               | 0.45   | g  |
| Na <sub>2</sub> S x 9 H <sub>2</sub> O          | 0.45   | g  |
| Distilled water                                 | 890.00 | ml |

Dissolve ingredients (except bicarbonate, glucose, vitamins and reducing agents), then sparge medium with 100% CO<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add solid bicarbonate, adjust pH to 6.8 - 7.0 and dispense medium under 100% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. The final pH of the medium should be 7.0.

For [DSM 14465](#), [DSM 14466](#): Supplement medium with 0.50 g/l sodium formate.

For [DSM 14469](#): Omit yeast extract from the medium and add 2.00 g/l Trypticase peptone (BD BBL), 2.50 g/l sodium acetate and 2.50 g/l sodium formate.

### **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 |

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| 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.

### Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N<sub>2</sub> gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

### 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 |