

## 503: ANAEROBIC FRESHWATER (FWM) MEDIUM

This recipe contains strain-specific modifications for *Clostridium homopropionicum* DSM 5847 \*

Final pH: 7.2 - 7.4

Final volume: 1003 ml

|                   |        |    |
|-------------------|--------|----|
| <b>Solution A</b> | 942.00 | ml |
| <b>Solution B</b> | 30.00  | ml |
| <b>Solution C</b> | 20.00  | ml |
| <b>Solution D</b> | 1.00   | ml |
| <b>Solution E</b> | 10.00  | ml |

Sparge solution A with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solution C and D are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Solution E is autoclaved under 100% N<sub>2</sub> gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.2 - 7.4, if necessary.

\* Replace D-glucose with 1.50 g/l of Na (D/L) 3 hydroxybutyrate or 2.00 g/l D-fructose as substrate.

### Solution A

|                                        |        |    |
|----------------------------------------|--------|----|
| KH <sub>2</sub> PO <sub>4</sub>        | 0.20   | g  |
| NH <sub>4</sub> Cl                     | 0.25   | g  |
| NaCl                                   | 1.00   | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O | 0.40   | g  |
| KCl                                    | 0.50   | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O | 0.15   | 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 |
| Distilled water                        | 940.00 | ml |

### Solution B

|                                 |       |    |
|---------------------------------|-------|----|
| Na <sub>2</sub> CO <sub>3</sub> | 1.50  | g  |
| Distilled water                 | 30.00 | ml |

### Solution C

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|                                             |                 |              |
|---------------------------------------------|-----------------|--------------|
| <del>D-Glucose</del>                        | <del>5.00</del> | <del>g</del> |
| Na-3-hydroxybutyrate (or 2.00 g D-fructose) | 1.50            | g            |
| Distilled water                             | 20.00           | ml           |

### Solution D

|                         |      |    |
|-------------------------|------|----|
| Seven vitamins solution | 1.00 | ml |
|-------------------------|------|----|

### Solution E

|                                        |       |    |
|----------------------------------------|-------|----|
| Na <sub>2</sub> S x 9 H <sub>2</sub> O | 0.30  | g  |
| Distilled water                        | 10.00 | ml |

### 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 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 |

### Seven vitamins solution (from medium 503)

|                                   |         |    |
|-----------------------------------|---------|----|
| Vitamin B <sub>12</sub>           | 100.00  | mg |
| p-Aminobenzoic acid               | 80.00   | mg |
| D-(+)-biotin                      | 20.00   | mg |
| Nicotinic acid                    | 200.00  | mg |
| Calcium pantothenate              | 100.00  | mg |
| Pyridoxine hydrochloride          | 300.00  | mg |
| Thiamine-HCl x 2 H <sub>2</sub> O | 200.00  | mg |
| Distilled water                   | 1000.00 | ml |