

## 1210a: THERMOSULFURIMONAS MEDIUM

| 10.00   | ~  |
|---------|--|
| 10.00   | g  |
| 4.00    | g  |
| 0.33    | g  |
| 0.33    | g  |
| 0.50    | g  |
| 1.00    | ml   |
| 1.00    | ml   |
| 0.50    | ml   |
| 1.00    | g  |
| 0.33    | g  |
| 1.50    | g  |
| 3.50    | g  |
| 0.20    | g  |
| 1.00    | ml   |
| 1000.00 | ml   |
|         | 0.33<br>0.33<br>0.50<br>1.00<br>1.00<br>0.50<br>1.00<br>0.33<br>1.50<br>3.50<br>0.20<br>1.00 |

Dissolve ingredients except carbonate, hydrogenphosphate, vitamins, acetate, thiosulfate and yeast extract, then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add hydrogenphosphate, vitamins, acetate, thiosulfate and yeast extract from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. The pH of the complete medium should be at 6.5 - 6.8.

For <u>DSM 29363</u>: Prepare medium without acetate, thiosulfate and yeast extract. Supplement medium after autoclaving with 2.50 g/l Fe(III)-citrate added from a sterile anoxic stock solution (10% w/v) prepared under 100% N<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 6.0.

For <u>DSM 100025</u>: Supplement medium with 0.50 g/l Na-pyruvate and replace thiosulfate with 0.50 g/l sodium pyrosulfite ( $Na_2S_2O_5$ ) added after autoclaving from freshly prepared anoxic stock solutions sterilized by filtration. Adjust pH of complete medium to 6.0 - 6.5.

For <u>DSM 100275</u>: Supplement medium after autoclaving with 1.00 g/l KNO<sub>3</sub> added from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas and 5.00 g/l sulfur (powdered) sterilized separately by steaming for 3 hours on each of 3 successive days.

For <u>DSM 104922</u>: Omit acetate and supplement medium after autoclaving with 1.00 g/l KNO<sub>3</sub> added from a sterile anoxic stock solution prepared under 100%  $N_2$  gas.

Trace element solution SL-10 (from medium 320)

## Microorganisms



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| HCI (25%)                      | 10.00  | ml |
|--------------------------------|--------|----|
| $FeCl_2 \times 4 H_2O$         | 1.50   | g  |
| ZnCl <sub>2</sub>              | 70.00  | mg |
| $MnCl_2 \times 4 H_2O$         | 100.00 | mg |
| H <sub>3</sub> BO <sub>3</sub> | 6.00   | mg |
| $CoCl_2 \times 6 H_2O$         | 190.00 | mg |
| $CuCl_2 \times 2 H_2O$         | 2.00   | mg |
| $NiCl_2 \times 6 H_2O$         | 24.00  | mg |
| $Na_2MoO_4 \ge H_2O$           | 36.00  | mg |
| Distilled water                | 990.00 | ml |

First dissolve  $\text{FeCl}_2$  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  |
| $Na_2SeO_3 \times 5 H_2O$                     | 3.00  | mg |
| $Na_2WO_4 \times 2 H_2O$                      | 4.00  | mg |
| Distilled water 10                            | 00.00 | 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 |