# 1011: SULFURIMONAS MJ MEDIUM

This recipe contains strain-specific modifications for *Thermotomaculum hydrothermale* DSM 24660 *

Final pH: * 6.5
Final volume: 1011 ml

| | | |
|---|---|---|
| NaCl | 30.00 | g |
| $K_2HPO_4$ | 0.14 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.14 | g |
| $MgSO_4$ x 7 $H_2O$ | 3.40 | g |
| $MgCl_2$ x 6 $H_2O$ | 4.18 | g |
| KCl | 0.33 | g |
| $NH_4Cl$ | 0.25 | g |
| $Fe(NH_4)_2(SO_4)_2$ x 6 $H_2O$ | 0.01 | g |
| **Modified Wolin's mineral solution** | 10.00 | ml |
| $NaHCO_3$ | 1.50 | g |
| ~~$Na_2S_2O_3$ x 5 $H_2O$~~ | ~~1.50~~ | ~~g~~ |
| **Wolin's vitamin solution (10x)** | 1.00 | ml |
| Yeast extract | 4.00 | g |
| $Na_2S$ x 9 $H_2O$ | 0.30 | g |
| Tryptone peptone | 4.00 | g |
| Distilled water | 1000.00 | ml |

1. Dissolve ingredients (except bicarbonate, thiosulfate and vitamins), then sparge medium with 80% $N_2$ and 20% $CO_2$ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add bicarbonate, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions. Solutions of vitamins and thiosulfate are sterilized by filtration and stored under $N_2$, whereas the solution of bicarbonate is prepared under 80% $N_2$ and 20% $CO_2$ gas mixture and autoclaved. Adjust pH of complete medium to 6.7.

2. After inoculation pressurize vessels to 0.5 bar overpressure with sterile 80% $N_2$ and 20% $CO_2$ gas mixture and add sterile air in an amount that is equivalent to a volume of 20% of the headspace.

* Omit thiosulfate and supplement medium with 4.00 g/l yeast extract and 4.00 g/l Trypton peptone. After autoclaving the medium is reduced with 0.30 g/l $Na_2S$ x 9 $H_2O$ added from a sterile anoxic stock solution (3% w/v) prepared under 100% $N_2$ gas and the pH adjusted to 6.5. After inoculation pressurize vessels to 1 bar overpressure with sterile 80% $N_2$ and 20% $CO_2$ gas mixture. Do not add sterile air!

## Modified Wolin's mineral solution (from medium 141)

| | | |
|---|---|---|
| Nitrilotriacetic acid | 1.50 | g |
| $MgSO_4$ x 7 $H_2O$ | 3.00 | g |
| $MnSO_4$ x $H_2O$ | 0.50 | g |
| NaCl | 1.00 | g |
| $FeSO_4$ x 7 $H_2O$ | 0.10 | g |
| $CoSO_4$ x 7 $H_2O$ | 0.18 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.10 | g |
| $ZnSO_4$ x 7 $H_2O$ | 0.18 | g |
| $CuSO_4$ x 5 $H_2O$ | 0.01 | g |
| $AlK(SO_4)_2$ x 12 $H_2O$ | 0.02 | g |
| $H_3BO_3$ | 0.01 | g |
| $Na_2MoO_4$ x 2 $H_2O$ | 0.01 | g |
| $NiCl_2$ x 6 $H_2O$ | 0.03 | g |
| $Na_2SeO_3$ x 5 $H_2O$ | 0.30 | mg |
| $Na_2WO_4$ x 2 $H_2O$ | 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 $B_{12}$ | 1.00 | mg |
| p-Aminobenzoic acid | 50.00 | mg |
| (DL)-alpha-Lipoic acid | 50.00 | mg |
| Distilled water | 1000.00 | ml |