# 825: METHANOBACTERIUM II MEDIUM

Final pH: 6.8 - 7.0
Final volume: 1011 ml

| | | |
|---|---|---|
| $NH_4Cl$ | 1.00 | g |
| $K_2HPO_4$ | 0.30 | g |
| $KH_2PO_4$ | 0.30 | g |
| $MgCl_2$ x 6 $H_2O$ | 0.20 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.10 | g |
| KCl | 0.10 | g |
| NaCl | 0.60 | g |
| **Modified Wolin's mineral solution** | 10.00 | ml |
| Na-acetate | 0.50 | g |
| Yeast extract | 1.00 | g |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| $NaHCO_3$ | 4.00 | g |
| **Wolin's vitamin solution (10x)** | 1.00 | ml |
| L-Cysteine HCl x $H_2O$ | 0.50 | g |
| $Na_2S$ x 9 $H_2O$ | 0.50 | g |
| Distilled water | 1000.00 | ml |

1. Dissolve ingredients except bicarbonate, vitamins, cysteine and sulfide. Sparge medium with 80% $H_2$ and 20% $CO_2$ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 7.0, then dispense medium under 80% $H_2$ and 20% $CO_2$ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% $N_2$ gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. After inoculation pressurize cultivation vessels to 2 bar overpressure with sterile 80% $H_2$ and 20% $CO_2$ gas mixture.

For DSM 24406: Supplement medium with 0.5 g/l DL-dithiothreitol (DTT), 20 ml/l methanol (50% v/v), and 0.5 g/l 2-mercaptoethanesulfonate (coenzyme M) added to the autoclaved medium from sterile anoxic stock solutions. The stock solution of DTT has to be sterilized by filtration. Omit $Na_2S$ x 9 $H_2O$ and L-cysteine HCl x $H_2O$. Adjust the pH of the final medium to 6.5.

## 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 |