# 321: METHANOCORPUSCULUM AGGREGANS MEDIUM

| | | |
|---|---|---|
| $NH_4Cl$ | 1.00 | g |
| $K_2HPO_4$ x 3 $H_2O$ | 0.40 | g |
| $MgCl_2$ x 6 $H_2O$ | 0.40 | g |
| **Minerals solution** | 50.00 | ml |
| Na-formate | 5.00 | g |
| Na-acetate | 1.00 | g |
| Trypticase peptone (BD BBL) | 1.00 | g |
| Yeast extract (OXOID) | 1.00 | g |
| **Modified Wolin's mineral solution** | 10.00 | ml |
| **Sludge fluid** | 5.00 | ml |
| **Clarified rumen fluid**, alternative (optional) | 5.00 | ml |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| $Na_2CO_3$ | 1.50 | g |
| L-Cysteine HCl x $H_2O$ | 0.20 | g |
| $Na_2S$ x 9 $H_2O$ | 0.20 | g |
| Distilled water | 935.00 | ml |

1. Dissolve ingredients except carbonate, cysteine and sulfide, then sparge medium with 80% $H_2$ 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 to 30% of their volume and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% $N_2$ gas and carbonate from a sterile anoxic stock solution prepared under 80% $N_2$ and 20% $CO_2$ gas mixture. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. After inoculation add sterile 80% $H_2$ and 20% $CO_2$ gas mixture to 2 bar overpressure.

## Minerals solution (from medium 317)

| | | |
|---|---|---|
| $KH_2PO_4$ | 6.00 | g |
| $(NH_4)_2SO_4$ | 6.00 | g |
| NaCl | 12.00 | g |
| $MgSO_4$ x 7 $H_2O$ | 2.60 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.16 | g |
| Distilled water | 1000.00 | ml |

## 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.

## **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% $N_2$ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

## **Sludge fluid** (from medium 119)

| | | |
|---|---|---|
| Yeast extract | 4.00 | g |
| Sludge | 1000.00 | ml |

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.