# 1043: IGNISPHAERA MEDIUM

| | | |
|---|---|---|
| $(NH_4)_2SO_4$ | 1.30 | g |
| $CaCl_2$ x 2 $H_2O$ | 74.00 | mg |
| $MgSO_4$ x 7 $H_2O$ | 0.28 | g |
| $KH_2PO_4$ | 0.28 | g |
| Yeast extract (OXOID) | 0.10 | g |
| Trypticase peptone (BD BBL) | 2.00 | g |
| **Modified Wolin's mineral solution** | 10.00 | ml |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| $FeCl_3$ x 6 $H_2O$ (0.1% w/v in 0.2 N HCl) | 0.50 | ml |
| L-Cysteine HCl x $H_2O$ | 0.30 | g |
| Starch (soluble) | 2.00 | g |
| $Na_2S$ x 9 $H_2O$ | 0.30 | g |
| Distilled water | 1000.00 | ml |

Dissolve ingredients (except ferric chloride, cysteine, starch and sulfide), sparge medium with 100% $N_2$ gas for 30 - 45 min to make it anoxic. Add ferric chloride and solid cysteine, then adjust pH to 6.3. Dispense medium under 100% $N_2$ gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Prior to inoculation add starch and sulfide from sterile anoxic stock solutions prepared under 100% $N_2$ gas. Adjust pH of complete medium to 6.5, if necessary.

## **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.