# 1577: SPOROHALOBACTER SALINUS MEDIUM

| | | |
|---|---|---|
| $NH_4Cl$ | 1.00 | g |
| $K_2HPO_4$ | 0.50 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.10 | g |
| KCl | 0.80 | g |
| Na-acetate | 0.50 | g |
| NaCl | 200.00 | g |
| Yeast extract (OXOID) | 0.50 | g |
| Tryptone (BD Bacto) | 5.00 | g |
| **Modified Wolin's mineral solution** | 10.00 | ml |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| L-Cysteine HCl x $H_2O$ | 0.50 | g |
| $MgSO_4$ | 5.00 | g |
| $Na_2CO_3$ | 1.00 | g |
| D-Glucose | 4.00 | g |
| $Na_2S$ x 9 $H_2O$ | 0.50 | g |
| Distilled water | 1000.00 | ml |

1. Dissolve ingredients (except cysteine, magnesium sulfate, carbonate, glucose and sulfide), then sparge medium with 80% $N_2$ and 20% $CO_2$ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve cysteine, adjust pH to 6.0, dispense under 80% $N_2$ and 20% $CO_2$ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add magnesium sulfate, glucose 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 atmosphere. Prior to inoculation check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. Note: This medium cannot be stored over a longer period and should be prepared freshly before use!

## 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.