# 1523: MODIFIED METHANOBACTERIUM MEDIUM

Final pH: 6.8 - 7.0
Final volume: 1002 ml

| | | |
|---|---|---|
| $KH_2PO_4$ | 0.50 | g |
| $MgSO_4$ x 7 $H_2O$ | 0.40 | g |
| NaCl | 0.40 | g |
| $NH_4Cl$ | 0.40 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.05 | g |
| **Trace element solution SL-10** | 1.00 | ml |
| Brain heart infusion (BD Bacto) | 6.00 | g |
| Proteose peptone (BD Difco) | 6.00 | g |
| Yeast extract (OXOID) | 2.00 | g |
| Na-acetate | 1.00 | g |
| Na-formate | 2.00 | g |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| $NaHCO_3$ | 4.00 | g |
| **Seven vitamins solution** | 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, then sparge medium with 80% $H_2$ and 20% $CO_2$ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 6.5. 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, cysteine, and sulfide from sterile anoxic stock solutions prepared under 100% $N_2$ gas. The vitamin solution should be sterilized by filtration. Before use, check the complete medium's pH and adjust it to 6.8 - 7.0, if necessary.

2. After growth has started and the culture is becoming turbid add sterile 80% $H_2$ and 20% $CO_2$ gas mixture to 0.5 - 1 bar overpressure.

For DSM 1093, DSM 1125, DSM 16643: Supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) and 0.30 g/l DTT (DL-Dithiothreitol) added from filter-sterilized anoxic stock solutions prepared under $N_2$. Omit sulfide and cysteine from the medium.

For DSM 1535: Adjust pH of complete medium to 7.6.

For DSM 2640, DSM 2702, DSM 3823, DSM 4140, DSM 4179, DSM 4273, DSM 4274, DSM 10196: Increase amount of Na-acetate to 4.00 g/l.

For DSM 15163: Adjust pH of complete medium to 6.0.

For DSM 16632: Supplement medium after autoclaving with 50 ml/l clarified rumen fluid

and 0.3 g/l DTT (DL-dithiothreitol). Omit $Na_2S$ x 9 $H_2O$.

For DSM 115764, DSM 115765: Supplement medium after autoclaving with 0.30 g/l DTT (DL-dithiothreitol), 10.00 ml/l methanol (50 % v/v), and 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from filter-sterilized anoxic stock solutions prepared under $N_2$. Omit $Na_2S$ x 9 $H_2O$ and L-cysteine HCl x $H_2O$.

## Trace element solution SL-10 (from medium 320)

| | | |
|---|---|---|
| HCl (25%) | 10.00 | ml |
| $FeCl_2$ x 4 $H_2O$ | 1.50 | g |
| $ZnCl_2$ | 70.00 | mg |
| $MnCl_2$ x 4 $H_2O$ | 100.00 | mg |
| $H_3BO_3$ | 6.00 | mg |
| $CoCl_2$ x 6 $H_2O$ | 190.00 | mg |
| $CuCl_2$ x 2 $H_2O$ | 2.00 | mg |
| $NiCl_2$ x 6 $H_2O$ | 24.00 | mg |
| $Na_2MoO_4$ x 2 $H_2O$ | 36.00 | mg |
| Distilled water | 990.00 | ml |

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

## Seven vitamins solution (from medium 503)

| | | |
|---|---|---|
| Vitamin $B_{12}$ | 100.00 | mg |
| p-Aminobenzoic acid | 80.00 | mg |
| D-(+)-biotin | 20.00 | mg |
| Nicotinic acid | 200.00 | mg |
| Calcium pantothenate | 100.00 | mg |
| Pyridoxine hydrochloride | 300.00 | mg |
| Thiamine-HCl x 2 $H_2O$ | 200.00 | mg |
| Distilled water | 1000.00 | ml |