

**334. METHANOTHRIX MEDIUM****Solution A:**

KH <sub>2</sub> PO <sub>4</sub>	0.22	g
Na <sub>2</sub> HPO <sub>4</sub> x 2 H <sub>2</sub> O	0.86	g
Na-acetate	6.80	g
Na <sub>2</sub> -EDTA solution (0.1% w/v)	1.00	ml
Trace elements solution (see medium 318)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	930.00	ml

**Solution B:**

NaHCO <sub>3</sub>	4.00	g
Distilled water	40.00	ml

**Solution C:**

NaCl	0.30	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.11	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
Distilled water	10.00	ml

**Solution D:**

NH <sub>4</sub> Cl	0.30	g
Distilled water	10.00	ml

**Solution E:**

Vitamins solution (see medium 141)	10.00	ml
------------------------------------	-------	----

**Solution F:**

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	10.00	ml

Sparge *solution A* with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic, dispense under same gas atmosphere into anoxic serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Prior to inoculation complete the medium by adding appropriate amounts of sterile *solutions C, D, E* and *F* prepared under 100% N<sub>2</sub> gas and *solution B* prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Vitamins are sterilized by filtration. Adjust the pH of the complete medium to 7.3 – 7.5.

*Note: Use 20% (v/v) inoculum.*

*Continued on next page*

For DSM 3870, DSM 4774 and DSM 6194 supplement medium with 10.00 ml/l of a coenzyme M (2-mercaptoethanesulfonate) solution (1.42% w/v, prepared under 100% N<sub>2</sub> gas) and adjust pH of complete medium to 7.0, if necessary.

For DSM 17206 supplement medium with 0.50 g/l yeast extract (OXOID) and 0.50 g/l Trypticase peptone (BD BBL) added from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere.