

**342. METHANOBACTERIUM ALCALIPHILUM MEDIUM****Solution A:**

NH <sub>4</sub> Cl	1.0	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.1	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.1	g
K <sub>2</sub> HPO <sub>4</sub>	0.4	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O solution (0.01% w/v)	2.0	ml
Trace element solution (see medium 141)	10.0	ml
Yeast extract (OXOID)	2.0	g
Trypticase peptone (BD BBL)	2.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.5	g
Distilled water	780.0	ml

**Solution B:**

NaHCO <sub>3</sub>	10.0	g
Na <sub>2</sub> CO <sub>3</sub>	0.5	g
Distilled water	210.0	ml

**Solution C:**

TRIS-HCl buffer 2 M (pH 8.4)	10.0	ml
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Dissolve ingredients of *solution A* (except cysteine), bring to the boil, then cool to room temperature under 100% H<sub>2</sub> gas. Add cysteine and adjust pH to 7.0, if necessary. Dispense under 100% H<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 3.9 ml medium per Hungate-type tube), then autoclave. *Solution B* is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. *Solution C* is prepared under 100% N<sub>2</sub> gas atmosphere. Complete the medium by adding appropriate amounts of *solutions B* and *C* to the sterile *solution A*. Adjust pH of complete medium to 8.3 - 8.4, if necessary. After inoculation add sterile H<sub>2</sub> gas to 1 - 2 bar overpressure.