

**1630. NITROSOSPHAERA MEDIUM**

NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
KH <sub>2</sub> HPO <sub>4</sub>	0.20	g
KCl	0.50	g
Na <sub>2</sub> SO <sub>4</sub>	0.05	g
HEPES buffer solution (see below)	10.00	ml
NH <sub>4</sub> Cl	0.05	g
Trace elements solution SL-11 (see medium 722)	1.00	ml
Na-pyruvate	0.05	g
NaHCO <sub>3</sub>	0.25	g
Wolin's vitamin solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except trace elements, HEPES buffer, ammonium chloride, pyruvate, bicarbonate and vitamins) and dispense medium under air atmosphere into culture vessels suitable for anaerobic cultivation (e.g., 20 ml medium per 100 ml serum bottle), seal with grey butyl rubber stoppers and autoclave. Add HEPES buffer and ammonium chloride from stock solutions sterilized by autoclaving. Trace elements, pyruvate, bicarbonate, and vitamins are added from sterile stock solutions sterilized by filtration. Adjust pH of the complete medium to 7.5 using a sterile solution of bicarbonate (5% w/v) or 1 N HCl.

*HEPES buffer solution:*

NaOH (pellets)	24.00	g
HEPES (free acid)	238.40	g
Distilled water	1000.00	ml

First dissolve NaOH in ca 600 ml water, then add HEPES, adjust pH to 7.5 and fill up to 1000 ml volume.