

## Main sol. 1630a

NaCl	26.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	5.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	5.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.50	g
KBr	0.10	g
KH <sub>2</sub> PO <sub>4</sub> (0.1% w/v)	2.00	ml
<b>HEPES buffer solution</b>	10.00	ml
NH <sub>4</sub> Cl	0.05	g
<b>Trace element solution SL-11</b>	1.00	ml
Na-pyruvate	0.05	g
NaHCO <sub>3</sub>	0.25	g
<b>Wolin's vitamin solution</b>	10.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except trace elements, HEPES buffer, ammonium chloride, pyruvate, bicarbonate and vitamins), dispense medium into Erlenmeyer flasks with screw caps (e.g., 20 ml medium per 100 ml flask) and autoclave. Add HEPES buffer and ammonium chloride from stock solutions sterilized by autoclaving. Trace elements, pyruvate, bicarbonate and vitamins are added from stock solutions sterilized by filtration. Adjust pH of the complete medium to 7.2 using sterile solutions of bicarbonate (5% w/v) or 1 N HCl.
2. Note: Use at least 5% (v/v) inoculum and incubate in the dark without shaking.