

## Main sol. J462

NaCl	20.00	g
Na <sub>2</sub> SO <sub>4</sub>	4.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	3.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
NH <sub>4</sub> Cl	0.75	g
KH <sub>2</sub> PO <sub>4</sub>	0.60	g
KBr	0.09	g
KCl	0.50	g
NaHCO <sub>3</sub>	2.50	g
<b>Trace element solution</b>	1.00	ml
<b>Vitamin solution</b>	1.00	ml
<b>Thiamine solution</b>	1.00	ml
<b>Vitamin B<sub>12</sub> solution 0.05 g/L</b>	1.00	ml
<b>Riboflavin solution 0.025 g/L</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium lactate	2.25	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Resazurin	1.00	mg

Dissolve components except NaHCO<sub>3</sub>, Vitamin solution, Thiamine solution, Vitamin B<sub>12</sub> solution, sodium lactate and Na<sub>2</sub>S x 9H<sub>2</sub>O in 900 ml distilled water and adjust pH to 7.0. Bring to a boil and cool down under a N<sub>2</sub>-CO<sub>2</sub> (4:1, v/v) gas stream. Distribute the medium into culture vessels (e.g., 9 ml of the medium in Hungate tubes) under a N<sub>2</sub>-CO<sub>2</sub> (4:1, v/v) gas mixture, seal with butyl rubber stoppers and autoclave. Separately autoclave 0.2 M sodium lactate under a N<sub>2</sub> atmosphere. After cooling, add 1/9 volume of the sodium lactate solution, and filter-sterilized 8% NaHCO<sub>3</sub> solution, Vitamin solution, Thiamine solution and Vitamin B<sub>12</sub> solution to the medium. Readjust pH to 7.0, if necessary. Prior to use, reduce the medium with Na<sub>2</sub>S x 9H<sub>2</sub>O (3% solution, autoclaved and stocked under N<sub>2</sub>).