



## Solution A

Na <sub>2</sub> SO <sub>4</sub>	0.700	g
K <sub>2</sub> HPO <sub>4</sub>	0.200	g
NH <sub>4</sub> Cl	0.250	g
NaCl	0.250	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.400	g
KCl	0.500	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.150	g
Resazurin	0.500	mg
Yeast extract	2.000	g
<b>FeCl<sub>2</sub> solution</b>	1.000	ml
<b>Trace element solution</b>	1.000	ml
<b>Selenite-tungstate solution</b>	1.000	ml
L-Cysteine HCl x H <sub>2</sub> O	0.050	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.025	g
Distilled water	900.000	ml

1. Mix components except L-cysteine x HCl x H<sub>2</sub>O and FeSO<sub>4</sub> x 7H<sub>2</sub>O. Bring to a boil for a few seconds and cool down under a N<sub>2</sub> atmosphere. While gassing with N<sub>2</sub>, add L-cysteine x HCl x H<sub>2</sub>O and FeSO<sub>4</sub> x 7H<sub>2</sub>O to the solution, distribute into culture vessels (e.g., 18 ml in 50 ml serum bottles) under a N<sub>2</sub> atmosphere, then, seal butyl rubber stoppers and autoclave. Aseptically and anaerobically add per liter (final) the following sterile solutions (autoclaved or \*filter-sterilized) from anaerobic stocks:

Potassium phosphate buffer (0.1 M), pH 7.5	10.000	ml
Trace vitamins (see Medium No. 197) (*)	10.000	ml
Vitamin B <sub>12</sub> solution (see Medium No. 403) (*)	1.000	ml
NaHCO <sub>3</sub> (*, 8%)	42.000	ml
Sodium pyruvate (*, 25%)	18.000	ml
Sodium fumarate (*, 16%)	40.000	ml

2. Adjust pH of the medium to 7.3 - 7.7, if necessary.