



Main sol. 1230

NaCl	6.00	g
K ₂ HPO ₄	2.00	g
NaHCO ₃	40.00	g
KNO ₃	0.50	g
Distilled water	1000.00	ml

1. Adjust the pH to 9.0 - 9.5 with 2M KOH. Dispense into closed vessels (i.e. a serum tube or bottle) with a liquid to air ratio of about 1:10. Flush the gas phase with nitrogen and add 2% oxygen. Sterilize at 120°C 20 min.

2. After sterilization add from sterile stock solutions:

Trace element solution	1.00	ml
MgCl ₂ x 6 H ₂ O (200.0 g/l)	1.00	ml

3. (a white colloid will form which will rapidly dissolve after mixing)

Sodium thiosulfate pentahydrate (4 M - 992 g/l)	5.00	ml
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4. Incubate statically until growth occurs, then shake at 100 rpm.