

## Main sol. 294

KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.25	g
NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
<b>Seven vitamins solution</b>	1.00	ml
Gentisic acid (2,5-Dihydroxybenzoic acid), sodium salt	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.10	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, gentisic acid and sulfide), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere in culture vessels and autoclave. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add gentisic acid (2,5-dihydroxybenzoic acid) from a sterile and neutralized 200 mM stock solution prepared under 100% N<sub>2</sub> gas atmosphere. If necessary, adjust the final pH of the medium to 7.2.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum. Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days. After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.