



## 197: JCM MEDIUM No. 197

Final pH: 7.0

Final volume: 1000 ml

KH <sub>2</sub> PO <sub>4</sub>	0.75	g
K <sub>2</sub> HPO <sub>4</sub>	0.75	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.36	g
NH <sub>4</sub> Cl	1.00	g
Yeast extract (BD-Difco)	0.10	g
Polypeptone (Nihon Pharm. Co.)	0.10	g
Sodium acetate	2.50	g
Trimethylamine-HCl	8.00	g
<b>Trace mineral solution</b>	9.00	ml
<b>Trace vitamins</b>	10.00	ml
NaHCO <sub>3</sub>	0.80	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Resazurin	1.00	mg
Distilled water	981.00	ml

Mix ingredients except NaHCO<sub>3</sub>, Trace vitamins, Na<sub>2</sub>S x 9H<sub>2</sub>O and cysteine x HCl x H<sub>2</sub>O, and autoclave under a N<sub>2</sub>-CO<sub>2</sub> (80:20) atmosphere. Filter-sterilize NaHCO<sub>3</sub> as an 8% solution and Trace vitamins while gassing with N<sub>2</sub>. Separately autoclave Na<sub>2</sub>S x 9H<sub>2</sub>O and cysteine x HCl x H<sub>2</sub>O as 5% solutions under a N<sub>2</sub> atmosphere. Aseptically and anaerobically add NaHCO<sub>3</sub> and Trace vitamins to the medium under a N<sub>2</sub>-CO<sub>2</sub> (80:20) atmosphere. Prior to inoculation, add the sterile Na<sub>2</sub>S x 9H<sub>2</sub>O and cysteine x HCl x H<sub>2</sub>O solutions, and adjust pH to 6.8.

### Trace vitamins

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium pantothenate	5.00	mg
Vitamin B <sub>12</sub>	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml



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**Trace mineral solution**

Nitrilotriacetic acid	4.500	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	0.400	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.100	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.170	g
ZnCl <sub>2</sub>	0.100	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.020	g
H <sub>3</sub> BO <sub>3</sub>	0.019	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.010	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.030	g
Distilled water	1000.000	ml

Adjust pH to 7.0 with 1 N KOH.