



272: JCM MEDIUM No. 272

Final volume: 1020 ml

KH ₂ PO ₄	0.40	g
NH ₄ Cl	0.90	g
MgCl ₂ x 6 H ₂ O	0.36	g
NaCl	10.00	g
Sodium acetate	0.50	g
Yeast extract (BD-Difco)	2.00	g
Trypticase peptone (BD-BBL)	2.00	g
Trace vitamins	10.00	ml
Trace element solution	10.00	ml
Na ₂ CO ₃	5.00	g
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Resazurin	1.00	mg
Distilled water	1000.00	ml

Mix ingredients, except Na₂CO₃, L-cysteine x HCl x H₂O and Na₂S x 9H₂O. Bring to a boil for 5-10 sec, then, cool down under a stream of H₂-CO₂ (80:20, v/v) and add Na₂CO₃. Dispense the medium into suitable culture vessels (e.g., 20 ml of the medium in 120 ml serum bottles) under a stream of H₂-CO₂ (80:20, v/v) and seal with butyl rubber stoppers. After autoclaving, stand the medium overnight. Separately autoclave cysteine x HCl and Na₂S x 9H₂O as 5% solutions, respectively, under a N₂ atmosphere. Prior to inoculation, aseptically and anaerobically add the L-cysteine x HCl x H₂O and Na₂S x 9H₂O solutions. Check pH of the medium to be around 7.5. Pressurize the inoculated bottles to 200 kPa H₂-CO₂ (80:20, v/v).

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 ₁₂	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml



272: JCM MEDIUM No. 272

Trace element solution

Nitrilotriacetic acid	12.800	g
FeCl ₃ x 6 H ₂ O	1.350	g
MnCl ₂ x 4 H ₂ O	0.100	g
CoCl ₂ x 6 H ₂ O	0.024	g
CaCl ₂ x 2 H ₂ O	0.100	g
ZnCl ₂	0.100	g
CuCl ₂ x 2 H ₂ O	0.025	g
H ₃ BO ₃	0.010	g
NaCl	1.000	g
NiCl ₂ x 6 H ₂ O	0.120	g
Na ₂ SeO ₃ x 5 H ₂ O	0.026	g
Distilled water	1000.000	ml

1. Dissolve nitrilotriacetic acid first in 200 ml of distilled water, and adjust pH to 6.5 with KOH. Then add the remaining salts and adjust volume to 1.0 L. Readjust pH to 7.0 with KOH.
2. Comment: For strain JCM 10134, incubate with a 10-20% inoculum.