

1526b: KIRITIMATIELLA (TYG) MEDIUM

Sea Salt (SIGMA)	60.00	g
Wolfe's mineral elixir	1.00	ml
Trypticase peptone (BD BBL)	1.00	g
Yeast extract (OXOID)	1.00	g
Glucose	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.

Wolfe's mineral elixir (from medium 792)

MgSO ₄ x 7 H ₂ O	30.00	g
MnSO ₄ x H ₂ O	5.00	g
NaCl	10.00	g
FeSO ₄ x 7 H ₂ O	1.00	g
CoCl ₂ x 6 H ₂ O	1.80	g
CaCl ₂ x 2 H ₂ O	1.00	g
ZnSO ₄ x 7 H ₂ O	1.80	g
CuSO ₄ x 5 H ₂ O	0.10	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.18	g
H ₃ BO ₃	0.10	g
Na ₂ MoO ₄ x 2 H ₂ O	0.10	g
(NH ₄) ₂ Ni(SO ₄) ₂ x 6 H ₂ O	2.80	g
Na ₂ WO ₄ x 2 H ₂ O	0.10	g
Na ₂ SeO ₄	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H₂SO₄, then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml