

683. TISSIERELLA (AMO.1) MEDIUM

NaCl	5.80	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.30	g
CaCl ₂ x 2 H ₂ O	0.15	g
NH ₄ Cl	0.27	g
KH ₂ PO ₄	0.20	g
Trace elements SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Yeast extract (OXOID)	10.00	g
Trypticase peptone (BD BBL)	0.50	g
L-Serine	2.00	g
L-Threonine	2.00	g
N-methylhydantoin (SIGMA)	5.64	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	4.50	g
L-Cysteine-HCl x H ₂ O	0.50	g
Vitamin solution (see medium 141)	10.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, cysteine and vitamins) and sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Adjust pH to 8.3 with 10 N NaOH, add bicarbonate and cysteine and dissolve. Switch gas phase to 80% N₂ and 20% CO₂ gas mixture and flush only the head space of the medium vessel. Dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave 15 min at 121°C. Add vitamins to the autoclaved medium from an anoxic stock solution prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 8.3, if necessary.

Note: If creatinine is used as the substrate replacing N-methylhydantoin, prepare separately an anoxic, filter sterilized stock solution of creatinine under 100% N₂ gas atmosphere.