



GROWTH MEDIA

9 - Modified version of ATCC 197 medium (TN)

Components	Concentration (g/L)
NaCl	10
KCl	0.1
MgCl ₂ · 6H ₂ O	0.2
NH ₄ Cl ₂	1
K ₂ HPO ₄	0.3
KH ₂ PO ₄	0.3
CaCl ₂ · 2H ₂ O	0.1
Cysteine-HCl	1
Yeast extract	2
Tryptone	2
Glucose (stock solution)	5
Resazurin (stock solution)	0.001
Vitamins solution * (stock solution)	
Trace element solution ** (stock solution)	

PROCEDURE:

Prepare the following stock solutions in distilled H₂O:

- Glucose 50%
- Resazurin 0.2%
- Vitamins solution *
- Trace element solution **

* To prepare the vitamins solution, dissolve the following components: p-aminobenzoic acid 5mg/L; nicotinic acid 5mg/L; D-Ca-pantothenate 5mg/L; pyridoxine-HCl 10mg/L; riboflavin 5mg/L; thiamine-HCl · 2H₂O 5mg/L; biotin 2mg/L; folic acid 2mg/L; lipoic acid 5mg/L; vitamin B₁₂ 0.10mg/L. Adjust the volume to 1L and sterilize by filtration (pore size 0.22 μM).

** To prepare the trace element solution, first dissolve nitrilotriacetic acid (1.5g/L), adjust the pH to 6.5 with KOH, then add the following components: MgSO₄ · 2H₂O 0.5g/L, NaCl 1g/L, FeSO₄·7H₂O 0.10 g/L, CoSO₄ · 7H₂O 0.18 g/L, CaCl₂ · 2H₂O 0.10g/L, ZnSO₄ · 7H₂O 0.18g/L, CuSO₄ · 7H₂O 0.010g/L, KAl(SO₄)₂ · 12H₂O 0.020g/L, H₃BO₄ 0.010g/L, Na₂MoO₄ · 2H₂O 0.010g/L, NiCl₂ · 6H₂O 0.025g/L, Na₂SeO₃ · 5H₂O 0.010g/L. Adjust the volume to 1L and the final pH to 7.0 with NaOH and autoclave at 121 °C for 20 min.

Dissolve the quantities of each component, except glucose, resazurin, vitamins and trace element solutions, listed in the table in the appropriate volume of distilled H₂O, taking into account the volume of other components, prepared as stock solutions, that must be added after sterilization. Before sterilization, adjust the pH to 7.0 with NaOH 1M. Autoclave the dissolved mixture at 121 °C for 20 min. After sterilization, add glucose, resazurin, vitamins (10ml/L) and trace element (10ml/L) solutions.