

558a: CLOSTRIDIUM (RB-9) MEDIUM

KH_2PO_4	1.50	g
$\text{Na}_2\text{HPO}_4 \times 12 \text{ H}_2\text{O}$	4.20	g
NH_4Cl	0.50	g
$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$	0.36	g
Na_2SO_4	1.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	10.00	g
Yeast extract	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, yeast extract, vitamin solution and reducing agents), adjust pH to 5 - 6 using HCl and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add glucose, yeast extract, vitamin solution, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 5.0 - 6.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$	3.00	g
$\text{MnSO}_4 \times \text{H}_2\text{O}$	0.50	g
NaCl	1.00	g
$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$	0.10	g
$\text{CoSO}_4 \times 7 \text{ H}_2\text{O}$	0.18	g
$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$	0.10	g
$\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$	0.18	g
$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$	0.01	g
$\text{AlK(SO}_4)_2 \times 12 \text{ H}_2\text{O}$	0.02	g
H_3BO_3	0.01	g
$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$	0.01	g
$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$	0.03	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	0.30	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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