

611: PYROBACULUM (BS) MEDIUM

Synthetic seawater mix	125.00	ml
Modified Wolin's mineral solution	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O (0.01% w/v)	2.00	ml
NH ₄ Cl	0.25	g
KH ₂ PO ₄	0.07	g
Sodium resazurin (0.1% w/v)	0.50	ml
KNO ₃	1.00	g
Yeast extract	0.50	g
NaHCO ₃	2.00	g
Distilled water	875.00	ml

Dissolve ingredients (except nitrate, yeast extract and bicarbonate) and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Adjust pH to 7.0 and dispense under 100% N₂ gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle). Replace gas phase with 80% H₂ and 20% CO₂ gas mixture and autoclave. Prior to inoculation add nitrate and yeast extract from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 7.0 - 7.2.

Synthetic seawater mix

NaCl	47.15	g
MgCl ₂ x 6 H ₂ O	18.10	g
MgSO ₄ x 7 H ₂ O	7.00	g
CaCl ₂ x 2 H ₂ O	3.13	g
Na ₂ SO ₄	3.24	g
KCl	1.20	g
Na ₂ CO ₃	0.10	g
NaBr	0.10	g
KBr	80.00	mg
SrCl ₂ x 6 H ₂ O	72.00	mg
H ₃ BO ₃	52.00	mg
Na ₂ HPO ₄	8.10	mg
NaF	2.40	mg
Na-silicate	0.40	mg
KI	50.00	µg
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g

MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ 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.