

1597b: NATRANAEROFABA MEDIUM

Solution A	500.00	ml
Solution B	500.00	ml
Solution C	1.00	ml
Solution D	1.00	ml
Solution E	5.00	ml
Solution F	10.00	ml
Solution G	5.00	ml
Solution H	10.00	ml
Solution I	10.00	ml

1. Sterilize solutions A and B in closed thick-walled screw-top bottles (e.g., SCHOTT) for 20 min at 120°C. The pH of solution A after sterilization should be 10. There is some precipitate forming that settles at the bottom after 3 - 4 days. It is best to remove precipitates by decantation before using solution A for medium preparation.
2. Combine solution A with solution B and sparge medium with 100% N₂ gas for at least 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions C to I are sterilized separately under 100% N₂ gas. Vitamins and pyruvate should be sterilized by filtration. To complete the medium appropriate amounts of solutions C to I are added to the combined sterile solutions A and B in the sequence as indicated. Final pH of the medium should be 9.5.
3. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.

Solution A

Na ₂ CO ₃	92.50	g
NaHCO ₃	17.50	g
NaCl	8.00	g
K ₂ HPO ₄	0.50	g
Distilled water	500.00	ml

Solution B

NaCl	120.00	g
K ₂ HPO ₄	1.25	g
KCl	2.50	g
Distilled water	500.00	ml

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Solution C

Trace elements solution (Pfennig & Lippert,1966) 1.00 ml

Solution D

Selenite-tungstate solution 1.00 ml

Solution E

NH ₄ Cl	0.20	g
MgSO ₄ x 7 H ₂ O	0.25	g
Distilled water	5.00	ml

Solution F

Na-pyruvate	2.20	g
Distilled water	10.00	ml

Solution G

Yeast extract	0.50	g
Distilled water	5.00	ml

Solution H

Wolin's vitamin solution 10.00 ml

Solution I

Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	10.00	ml

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
FeSO ₄ x 7 H ₂ O	2.20	g
ZnSO ₄ x 7 H ₂ O	0.10	g
MnCl ₂ x 4 H ₂ O	0.03	g
H ₃ BO ₃	0.03	g
CoCl ₂ x 6 H ₂ O	0.20	g
CuCl ₂ x 2 H ₂ O	0.03	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B ₁₂	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml