

PAN2 (10 mM acetate, 5 mM Nitrate, 0.05 g/L YE)

For 500ml PAN2:

5ml 100x salt stock
5ml 100x phosphate stock
1.25ml trace minerals
2.5ml DL vitamin
5ml PIPES stock solution (1.0M, pH 7.0)
0.68 g sodium acetate trihydrate
0.21 g sodium nitrate
0.025 g yeast extract

100x salt solution stock:

	<u>g/100ml</u>
CaCl ₂	0.68
KCl	0.15
MgCl ₂	0.5
NH ₄ Cl	0.54

100x phosphate solution stock

	<u>g/100ml</u>
KH ₂ PO ₄	0.136

Trace Mineral Solution (SL 10)

Dist. H ₂ O	1000 ml
Conc. HCl	2 ml
MnCl ₂ · 6 H ₂ O	100 mg
CoCl ₂ · 6 H ₂ O	190 mg
ZnSO ₄ · 7 H ₂ O	144 mg
H ₃ BO ₃	6 mg
NiCl ₂ · 6 H ₂ O	24 mg
CuCl ₂ · 2 H ₂ O	2 mg
Na ₂ MoO ₄ · 2 H ₂ O	36 mg

Vitamin stock solution

Dist. H ₂ O	1000 ml
Biotin	2 mg
Folic acid	2 mg
Pyridoxine HCl	10 mg
Thiamine HCl	5 mg
Riboflavin	5 mg
Nicotinic acid	5 mg
DL Ca Pantothenate	5 mg
Vitamin B ₁₂	0.1 mg
P-aminobenzoic acid	5 mg
Lipoic acid	5 mg

PAN2 Medium Preparation:

- 1) Into a 1-L flask, add 100x salt stock, 100x phosphate stock, trace minerals, and 1.0M PIPES solution as described above. Add deionized water to 500mL mark. Put in stir bar and bring to a boil.
- 2) After autoclaving, cool the bottle under a stream of N₂, He or Ar while stirring (use stoppers and cannulas here). While cooling, add acetate, nitrate, vitamins, and yeast extract. After cooling, adjust pH to 6.9 to 7.1 with 0.1N HCl or NaOH if needed.
- 3) After cooling, stopper tightly to maintain anoxic conditions and transfer to the anaerobic chamber. The completed medium can be dispensed into serum bottles or Balch tubes in the anaerobic chamber or using anaerobic techniques outside the chamber.