

**TAP medium:**

Per 1000 mL:  
10 mL 5 x Beijerincks  
8.33 mL phosphate solution  
10 mL Tris-Acetate stock\*  
1 mL Trace Elements stock

Or (*recommended*)

10 mL 5 x Beijerincks or 50 ml 1 x Beijerincks  
8.33 mL phosphate solution  
10 mL Tris -HCl\*  
1 mL Trace Elements stock  
+ 1 mL Acetic Acid (Glacial)

**Minimal medium:**

Per 1000 mL:  
10 mL 5 x Beijerincks  
8.33 mL phosphate solution  
10 mL 2 M Tris base  
1 mL Trace Elements stock solution  
About 1.1 mL concentrated HCl to adjust pH to 6.8-7.2

**Stock Solutions:** (hereafter, molarities indicated are the final concentrations in TAP)

**5 x Beijerinck's Salt Stock:**

For 1000 mL  
NH<sub>4</sub>Cl 40 g (7.5 mM)  
CaCl<sub>2</sub> (2H<sub>2</sub>O) 5 g (0.34 mM)  
MgSO<sub>4</sub> (2H<sub>2</sub>O) 10 g (0.64 mM)

- Dissolve CaCl<sub>2</sub> in 300 mL H<sub>2</sub>O This takes some time to go into solution..
- Dissolve NH<sub>4</sub>Cl and MgSO<sub>4</sub> in 500 mL.
- Mix both solutions and add H<sub>2</sub>O to 1000 mL.
- If low sulphate medium is desired, replace MgSO<sub>4</sub> (2H<sub>2</sub>O) with 8.15 g MgCl<sub>2</sub> (6H<sub>2</sub>O).
- For anhydrous MgSO<sub>4</sub> measure 4.82 g.

**Phosphate Stock:**

For 1000 mL (Concentration of phosphate):  
K<sub>2</sub>HPO<sub>4</sub> (anhydrous) 14.34 g (0.68 mM)  
KH<sub>2</sub>PO<sub>4</sub> (anhydrous) 7.26 g (0.45 mM)

Check pH shall around pH to 7.1 (adjust with KOH (20% W/V) or similar).

**Tris(-Acetate) Stock Solution:**

For 1000 mL  
Tris base 242 g (20 mM)  
Glacial Acetic Acid 100 mL (17 mM)

Or (*recommended*)

Replace Acetic Acid with 1 mL HCl and titrate to pH ~6.6

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**Trace Elements Solution** (Hutner 1946, J. Bact. 52: 213):

for 1000 mL

EDTA-Na<sub>2</sub> 50.0 gH<sub>3</sub>BO<sub>3</sub> (boric acid) 11.14 gZnSO<sub>4</sub> • 7H<sub>2</sub>O 22.0 gMnCl<sub>2</sub> • 4H<sub>2</sub>O 5.1 gFeSO<sub>4</sub> • 7H<sub>2</sub>O 5.0 gCoCl<sub>2</sub> • 6H<sub>2</sub>O 1.6 gCuSO<sub>4</sub> • 5H<sub>2</sub>O 1.6 g(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> • 4H<sub>2</sub>O 1.1 g

- Add elements one at a time (except EDTA) to 550 mL milliQ H<sub>2</sub>O in a 1L Erlenmeyer flask.
  - Heat up solution to approximately 70 °C.
  - In a different beaker, add EDTA to 250 mL milliQ H<sub>2</sub>O and heat until dissolved.
  - Add EDTA solution to salt solution (not vice versa) and bring the combined solution to a boil.
  - Let solution cool down and maintain temperature at 70 – 75 °C.
  - Adjust pH to 6.5 – 6.8 with 20% KOH. Be careful: do not let the temperature fall below 70 °C or let the pH go over 6.8 otherwise you have to start all over again!
  - Dilute to 1000 mL.
  - Use a cotton plug to cover flask (not parafilm!) and let it stand for two weeks until the colour changes from green to purple. Stir solution to help it, all along.
  - Filter out red-brown precipitate and store in refrigerator. Note if there is a precipitate, then you don't know the real concentrations of elements in your solution.
  - Use 1 mL per 1000 mL medium.
  - For a trace element solution lacking a specific metal, leave out the appropriate metal. The solution will have a different coloration and only a little or no precipitate. Copper free trace elements solution for example will be reddish instead of purple.
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