Leucine and Phospahte Limiting Media

Media and growth conditions: Chemically defined media were based on (Saldanha et al. 2004), with modifications to chemically complement auxotrophies present in the deletion collection strain. For both phosphate and leucine limiting media we used

- 5.0g/L (NH4)2SO4,
- 0.50g/L MgSO4.7H2O,
- 0.10g/L CaCl2.2H2O,
- 0.10g/L NaCl,
- 40mg/L histidine,
- 40mg/L uracil,
- 60mg/L lysine.

For phosphate limiting media we added 200mg/L leucine and 1.0g/L KCl.
The only source of phosphorous was KH2PO4, which was present at an initial concentration of 5mg/L.
For leucine limiting media we added 1.0g/L KH2PO4 and 20mg/L leucine.

**Phosphate limiting media (1L)**

1. Prepare 10X salts for phosphate limitation (1L):
   * 1 g Calcium Chloride : CaCl2.2H2O
   * 1 g Sodium Chloride : NaCl
   * 5 g Magnesium Sulfate : MgSO4.7H2O
   * 50 g Ammonium Sulfate : (NH4)2SO4
   * 1 g Potassium Chloride : KCl
   * dH2O to 1000 ml, and then autoclave:

2. For 1L Phosphate limiting media (1X):
   * 100 ml of 10X phosphate limitation salts (final = 1X)
   * X1 ml of 4 g/L histidine (final = 40 mg/L)
   * X2 ml of 2 g/L uracil (final = 40 mg/L)
   * X3 ml of 10 g/L methionine (final = 40 mg/L)
   * X4 ml of 6 g/L lysine (final = 60 mg/L)
   * X5 ml of 10 g/L leucine (final = 200 mg/L)
   * \( (847.5 - (X1+X2+X3+X4+X5)) \) ml dH2O
   * Total volume = 947.5 ml, and then autoclave:

   # The addition of amino acids should be referred to auxotrophic genotypes of strains to be used.

3. Finally add:
   * 0.5 ml of 10 g/L potassium phosphate, KH2PO4 (final = 5 mg/L) : The only source of phosphorous
   * 50 ml 40 % glucose (final = 2 %)
   * 1 ml 1000X vitamins (final = 1X)
   * 1 ml 1000X metals (final = 1X)