**Recipe for optimized *C. reinhardtii* production medium by** [**Freudenberg *et al*. 2021**](https://www.sciencedirect.com/science/article/pii/S0960852420318162?via%3Dihub)

Optimized phototrophic production medium (6xP) for high cell density cultivation of nitrate capable *C. reinhardtii*. The medium is based on the original TAP medium recipe (Gorman & Levine, 1965) but avoids the components Tris, acetate and ammonium. The respective micro nutrient composition is described in Kropat *et al.* (2011) with Na2MoO4 used. The pH stabilizes around ~7.1 in ambient air and does not drop below 6.5 in CO2-enriched conditions. In stationary growth phase, removing CO2-gassing results in a pH of about 9. Note: Only nitrate and CO2 serve as a nitrogen / carbon source. Ensure adequate light regimes (>700 µmol/m2/s) and CO2 (>3% (v/v)) availability for optimal growth. To avoid calcium phosphate precipitation, add the P-solution after autoclaving.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **component** | **25x stock** | | **1x final (in medium)** | |
| MgSO4 ∙ 7 H2O | 60 mM | 14.8 g/L | 2.4 mM | 591 mg/L |
| CaCl2 ∙ 2 H2O | 30 mM | 4.4 g/L | 1.2 mM | 176 mg/L |
| NaNO3 | 1125 mM | 95.6 g/L | 45.0 mM | 3824 mg/L |

1. **Prepare 25x stock salt solution**
2. **Prepare 500x stock P-solution**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **component** | **500x stock** | | **1x final (in medium)** | |
| K2HPO4 | 2.2 M | 383.2 g/L | 4.4 mM | 766 mg/L |
| KH2PO4 | 0.9 M | 122.5 g/L | 1.8 mM | 244 mg/L |

1. **Prepare Kropat’s trace elements solutions 1-7**

Prepare all 7 trace elements stock solutions as described in Kropat *et al.* (2011) but replace stock solution 2 (28.5 µM (NH4)6Mo7O24) with 200 µM Na2MoO4 to avoid ammonium. Use them as 333x stock (<https://doi.org/10.1111/j.1365-313X.2011.04537.x>).

1. **Prepare final medium as follows:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Component | 1 L | 2 L | 3 L | 4 L | 5 L |
| Salt solution | 40 mL | 80 mL | 120 mL | 160 mL | 200 mL |
| Trace elements 1-7, each | 3 mL | 6 mL | 9 mL | 12 mL | 15 mL |

No pH adjustment required. Autoclave and cool down. Add 2 mL of autoclaved 500x P-solution per liter medium under sterile conditions. Small amounts of precipitate may form after a few days. This will not affect cell growth.