

NIES-C medium preparation

Haematococcus pluvialis was cultured using NIES-C medium, comprised of the following (per liter): 0.64 mM $\text{Ca}(\text{NO}_3)_2$, 0.99 mM KNO_3 , 0.23 mM β -glycerophosphoric acid disodium salt pentahydrate, 0.16 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4.13 mM Tris-aminomethane, 0.03 μM thiamine, 4.09×10^{-4} μM biotin, and 7.38×10^{-5} μM vitamin B_{12} , along with 3 mL PIV solution. The PIV metal solution consisted of 8.06 mM ethylenediaminetetraacetic acid disodium salt dihydrate, 2.18 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.55 mM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.23 mM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 mM $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, and 0.03 mM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. After adjusting the pH to 7.5, the NIES-C medium was sterilized using a 0.2 μm filter.