



TABLE 2. *Composition of freshwater medium WC.*

	mg/liter		$\mu\text{M}$
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	36.76		250
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	36.97		150
$\text{NaHCO}_3$	12.60		150
$\text{K}_2\text{HPO}_4^{\text{a}}$	8.71		50
$\text{NaNO}_3^{\text{a}, \text{b}}$	85.01		1000
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	28.42		100
Trace metals <sup>c, d</sup>	mg/liter compound	weight/liter of element	$\mu\text{M}$ element
$\text{Na}_2 \cdot \text{EDTA}^{\text{e}}$	4.36	—	ca. 11.7 (EDTA)
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}^{\text{e}}$	3.15	0.65 mg Fe	ca. 11.7
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.01	2.5 $\mu\text{g}$ Cu	ca. 0.04
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.022	5.0 $\mu\text{g}$ Zn	ca. 0.08
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.01	2.5 $\mu\text{g}$ Co	ca. 0.05
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.18	0.05 mg Mn	ca. 0.9
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.006	2.5 $\mu\text{g}$ Mo	ca. 0.03
$\text{H}_3\text{BO}_3$	1.0	0.17 mg B	ca. 16
<b>Vitamins</b>			
Thiamin $\cdot$ HCl	0.1 mg/liter		
Biotin	0.5 $\mu\text{g}$ /liter		
$\text{B}_{12}$	0.5 $\mu\text{g}$ /liter		

Buffers: Glycylglycine at 500 mg/liter (3.79 mM) is used for axenic cultures. For cultures with bacteria, Tris at 500 mg/liter (4.1 mM) is used. Adjust pH as desired, usually 7–8.

<sup>a</sup> Nitrate and phosphate should be reduced 2–10-fold for sensitive organisms.

<sup>b</sup> Use  $\text{NH}_4\text{Cl}$ , 2.65–26.5 mg/liter (50–500  $\mu\text{M}$ ) for organisms that cannot utilize nitrate.

<sup>c</sup> The more complete trace metal solution using ferric citrate-citric acid described by Wright (16) can also be used.

<sup>d</sup> The pH of the trace metal solution is best adjusted to 4–4.5 as a compromise to retain solubility of the metals in a  $\times 1000$  stock solution yet not lower the pH of the final nutrient solution too much.

<sup>e</sup> Ferric citrate-citric acid 3–9 mg/liter each can be used in place of ferric EDTA.