"Special K" Trace Elements Recipe devised by Merchant group (Janette Kropat and Davin Malasarn) – work is unpublished, details of development and analysis will follow. Please do not distribute outside the NAABB consortium until after publication.

Revised June 2, 2010

Make preliminary concentrated stock solutions in Part A first, and, where indicated, use these to make the individual stock solutions in Part B listed below. Only solutions in Part B are added directly to media.

## A. Preliminary concentrated stock solutions

Pre-1. EDTA-Na$_2$ concentrate      125 mM      13.959 g in ~ 250 ml, titrate to pH 8.0 with trace element grade KOH (~1.7 g), and bring up to a volume of 300 ml

Pre-2. $(NH_4)_6Mo_7O_{24}$ concentrate      285 µM      $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 0.088 g, bring up to a volume of 250 mL

Pre-3. $Na_2SeO_3$ concentrate      1 mM      $Na_2SeO_3$: 0.043 g, bring up to a volume of 250 mL

## B. Individual Stock Solutions for medium (1000×)

Bring each stock solution up to 250 mL in water. Use 1 mL of each individual stock solution in 1 L medium.

| Stock Solution | Concentration in stock | Composition |
| --- | --- | --- |
| 1. EDTA-Na$_2$ | 25 mM | EDTA-Na$_2$: 50 mL of 125 mM EDTA-Na$_2$ concentrate (Pre-1) from Step A |
| 2. $(NH_4)_6Mo_7O_{24}$ | 28.5 µM* | $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 25 mL of 285 µM $(NH_4)_6Mo_7O_{24}$ concentrate (Pre-2) from Step A |
| 3. $Na_2SeO_3$ | 0.1 mM | $Na_2SeO_3$: 25 mL of 1 mM $Na_2SeO_3$ concentrate (Pre-3) from Step A |
| 4. Zn·EDTA | 2.5 mM<br>2.75 mM | $ZnSO_4 \cdot 7H_2O$: 0.18 g<br>EDTA-Na$_2$: 5.5 mL of 125 mM EDTA-Na$_2$ concentrate (Pre-1) from Step A |
| 5. Mn·EDTA | 6 mM<br>6 mM | $MnCl_2 \cdot 4H_2O$: 0.297 g<br>EDTA-Na$_2$: 12 mL of 125 mM EDTA-Na$_2$ concentrate (Pre-1) from Step A |

| 6. Fe·EDTA | 20 mM | $FeCl_3 \cdot 6H_2O$: 1.35 g |
| | 22 mM | EDTA-Na$_2$: 2.05 g |
| | 22 mM | $Na_2CO_3$ (sodium carbonate): 0.58 g |

(Combine EDTA-Na$_2$ with sodium carbonate in water and mix. Add $FeCl_3 \cdot 6H_2O$ after the first two components dissolve. Do Not Use Pre-1.)

| 7. Cu·EDTA | 2 mM | $CuCl_2 \cdot 2H_2O$: 0.085 g |
| | 2 mM | EDTA-Na$_2$: 4 mL of 125 mM EDTA-Na$_2$ concentrate (Pre-1) from Step A |

Notes:
*The final [Mo] in the 1× medium is 0.2 µM
Total [EDTA] in 1× medium: $(25 + 2.75 + 6 + 22 + 2) = 57.75$ µM