

A. EQUIPMENT

UV/Vis spectrometer	Vortex mixer
Water bath (stable at 40°C)	Microcentrifuge and 1.5 mL tubes
Glassware	Micro-cuvettes (1.5 mL)
Analytical balance	Timer
Pipettes (20 µL to 5 mL)	Megazyme calculation software

B. REAGENTS

Megazyme® phytic acid assay kit	Powdered ascorbic acid
Concentrated Sulfuric acid	Sodium hydroxide pellets
Hydrochloric acid	Powdered ammonium molybdate
Powdered trichloroacetic acid	

C. STABILITY OF REAGENTS

The Megazyme phytic acid assay kit provided solutions stable for over two years at 4°C:

Two buffer solutions (pH 5.5 and 10.4)	Phytase suspension
Alkaline phosphatase	Phosphorus standard solution
Oat flour reference material	

Other reagent solutions not supplied included the following,

Ascorbic acid (10% w/v)/Sulfuric acid (1 M)	Stable for one week at 4°C
Ammonium molybdate (5% w/v)	Stable for one month at 4°C
Trichloroacetic acid (50% w/v)	Stable for 6 months at 4°C
Hydrochloric acid (0.66 M)	Stable at room temperature
Sodium hydroxide (0.75 M)	Stable at room temperature

The supplied phosphorus standard was used to prepare a five phosphorus concentrations from 0 to 7.5 µg of phosphorus for calibration, including DI water, which were stable for one week at 4°C. The method employs a colour change in sample solutions as a result of the reaction of ascorbic acid and ammonium molybdate solutions in a 5:1 ratio. Due to its instability the complex/sample was prepared on the day of analysis ([Appendix 1](#)).

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