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Validation Report: Phytic Acid (Total Phosphorus) Assay Kit (cat. no. K-PHYT)

1. Scope

Megazyme's Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT) is a colourimetric method used for the measurement and analysis of phytic acid in cereal products, seed materials, feeds and foodstuffs. This method is a novel method developed in-house and measures phytic acid and total phosphorus in g/100 g.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT).

3. Performance characteristics

The selectivity, working range, limit of detection, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

This assay is specific for the measurement of phosphorus released as "available phosphorus" from phytic acid, *myo*-inositol (phosphate)_n and monophosphate esters by phytase and alkaline phosphatase.

This kit does not measure *myo*-inositol in either its free or phytase/alkaline phosphatase released forms.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding phytic acid to the sample at the initial extraction steps.



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3.2. Working Range

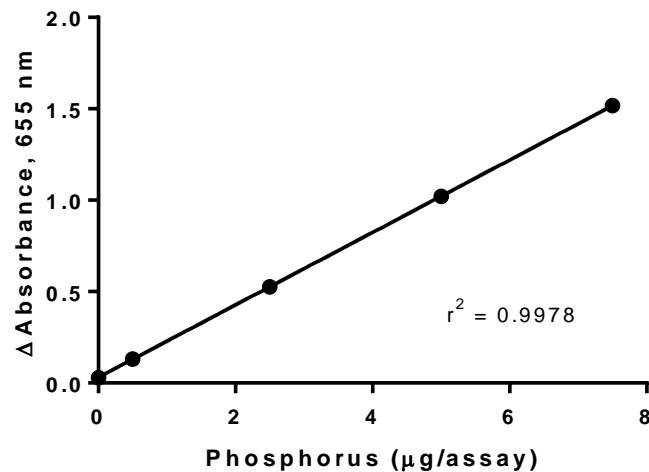
Assay follows the Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT) standard procedure.

The linearity of the phytic acid assay, comprising A) Sample Extraction, B) Enzymatic Dephosphorylation Reaction and C) Colourimetric Determination of Phosphorus assay is dictated by the dynamic ranges of the Enzymatic Dephosphorylation Reaction and the Colourimetric Determination of Phosphorus assays.

The working range for the Colourimetric Determination of Phosphorus assay was determined from a phosphorus calibration curve ranging from 0-7.5 $\mu\text{g}/\text{mL}$ using the phosphorus standard solution (50 $\mu\text{g}/\text{mL}$) provided in the kit. Linearity was observed up to a phosphorus concentration of 7.5 $\mu\text{g}/\text{mL}$

Phosphorus Concentration [$\mu\text{g}/\text{assay}$]	$\Delta A_{510\text{nm}}$
0	0.0700
0.5	0.1700
2.5	0.5700
5.0	1.0690
7.5	1.5620

Linearity of Colourimetric Determination of Phosphorus



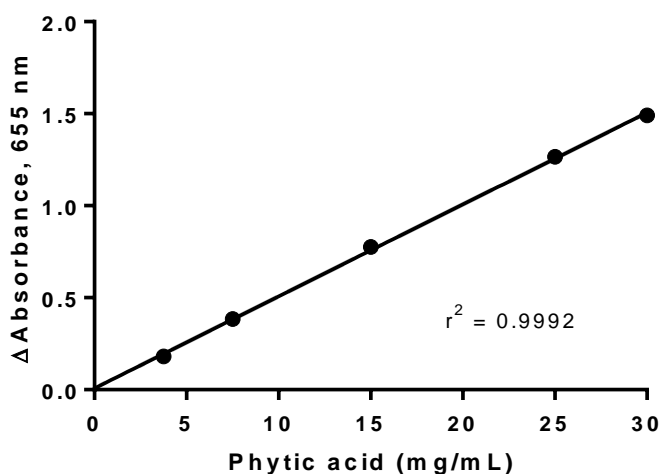


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The linearity of the Enzymatic Dephosphorylation Reaction was assessed using phytic acid solutions ranging from 3.75-30 mg/mL. Linearity was observed up to 30 mg/mL. In the standard phytic acid assay procedure this equates to a maximum phytic acid content of 3.0 g phytic acid/100g when 1 g of solid sample is applied to the assay.

Phytic Acid Concentration [mg/mL]	ΔA_{655nm}
3.75	0.1813
7.5	0.3845
15	0.7757
25	1.2655
30	1.4907

Linearity of Enzymatic Dephosphorylation Reaction





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3.3. LOD

The **instrument limit of detection** is ~ 11.29 mg phosphorus/100 g of original sample (or ~ 40 mg phytic acid/100 g), which is derived from an absorbance difference of 0.020, under the conditions of the standard assay procedure as stated in the Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT) kit data booklet.

The **calculated limit of detection (LOD)** and the **calculated limit of quantification (LOQ)** for purpose of this report is based on the analysis of samples that have been taken through the whole Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT) measurement procedure.

- LOD and lower LOQ were calculated as $3 \times \sigma$ of the blank sample solution absorbance and $10 \times \sigma$ of the blank sample solution absorbance, respectively, using absorbance values from 20 replicates.
- For Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT)

LOD

Phytic Acid = 0.09 g of phytic acid /100 g

LOQ

Phytic acid = 0.31 g of phytic acid/100 g

* **Note:** The above detection limits are for samples as used in the assay, after sample preparation. The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.



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3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT) method with a suitable reference value (x_{ref}). For this report, Relative Bias is calculated in percent as: $b(\%) = \frac{x - x_{ref}}{x_{ref}} \times 100$. The reference material for this purpose is a milled oat flour preparation that is supplied with the Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT), at 1.9 g of phytic acid/100 g.

Relative Bias $b(\%)$

	n	Ref Material (g/100 g)	Mean (g/100 g)	$b(\%)$
Phytic Acid	25	1.9	1.94	2.10

3.5. Precision

This report details the repeatability (intra-assay precision) and intermediate precision (inter-assay precision) of the Phytic Acid (Total Phosphorus) Assay Kit (K-PHYT). It is a measure of the variability in results, using 5 replicate samples of milled oat flour, in 4 separate phytic acid assay experiments.

Replicate	Phytic acid (g/100 g)				Intermediate Precision (%CV)
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	
1	1.916	1.965	1.932	1.824	
2	1.915	1.961	1.931	1.935	
3	1.959	1.930	1.905	1.839	
4	1.945	1.935	1.925	1.861	
5	1.916	1.942	1.844	1.851	
MEAN	1.930	1.947	1.908	1.862	1.91
STDEV	0.020	0.016	0.037	0.043	0.04
Repeatability (%CV)	1.06	0.81	1.94	2.32	2.27



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3.6. Sample analysis, recovery and specificity

A variety of nine milled food or feed samples were applied to the phytic acid assay in duplicate, with and without phytic acid spiking. The samples used were barley flour, malt flour, oat flour, wheat flour, American long grain rice, Odlum's cream plain flour, Kellogg's All-Bran Original cereal, pinto beans and red lentils. Spiked samples were supplemented with 7.5 mg phytic acid, which was added before the extraction of phytic acid procedure.

Sample Id	Phytic acid (mg)		Accuracy (% Recovery)
	Sample only	Spiked sample	
Barley Flour	4.3	11.8	100
Malt Flour	5.3	12.7	99
Oat Flour	18.2	26.7	112
Wheat Flour	1.1	8.4	97
American Long Grain Rice	1.9	9.6	102
Odlum's Cream Plain Flour	2.6	11.2	115
Kellogg's Original "All Bran"	15.6	23.7	107
Pinto Beans	8.9	17.3	112
Red Lentils	6.4	14.6	109
Phytic acid	7.5		

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of phytic acid in food and feed samples. Data presented in this report verifies and validates that this method is fit for the purpose intended and is summarised below.

Validation Summary	Phytic Acid
Working range (mg/mL)	Up to 30
LOD (g/100 g)	0.09
LOQ (g/100 g)	0.31
Relative Bias <i>b</i> (%)	2.10
Repeatability (%CV using oat flour)	2.27
Recovery Rates (%)	97-115

5. References

McKie, V. & McCleary, B. (2016). A Novel and Rapid Colorimetric Method for Measuring Total Phosphorus and Phytic Acid in Foods and Animal Feeds. *Journal of AOAC International*, 99(3), 738-743.