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**Validation Report: α -Amylase SD Assay Kit (High Sensitivity Method)
(cat. no. K-AMYLSD)**

1. Scope

Megazyme's α -Amylase SD Assay Kit (High Sensitivity Method) (K-AMYLSD) is a highly sensitive colourimetric method used for the rapid measurement and determination of α -amylase in sprout damaged wheat grain and food products. This α -amylase SD method was developed in-house and measures α -amylase as α -Amylase SD U/g. This method is based on AOAC (Method 2002.01), AACC (Method 22-02.01) and ICC (Standard No. 303).

2. Planning

The purpose of this report is to verify and validate the current method as detailed by α -Amylase SD Assay Kit (K-AMYLSD).

3. Performance characteristics

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

3.1. Selectivity

This assay is specific for α -amylase.

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 α -amylase to the sample in the initial extraction steps.

3.2. Working Range

The working range of the α -Amylase Assay Kit (K-AMYLSD) is up to ~ 0.5 α -Amylase SD U/g based on the standard assay procedure (0.4 mL α -amylase plus 0.1 mL Amylase SD Reagent with an incubation time of 10 min) and a maximum absorbance of 1.5.

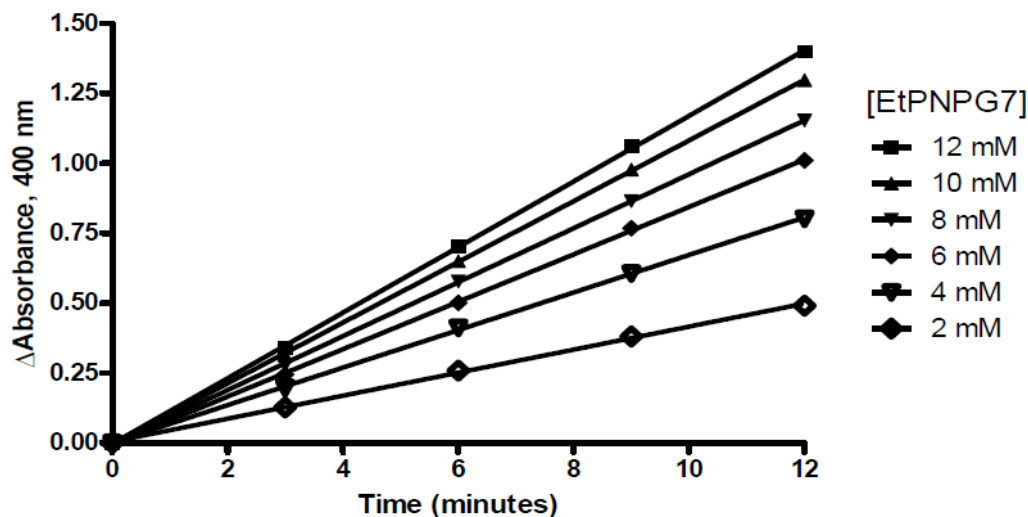
The linearity of the assay was assessed using a wheat α -amylase and various concentrations of substrate (2-12 mM EtPNPG7). Following the standard K-AMYLSD assay procedure, 0.4 mL of α -amylase was incubated at 40°C with 0.1 mL of Amylase SD substrate solution. The reactions were terminated at 2 min intervals and the absorbance values read at 400 nm.



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Linearity of the α -Amylase SD Assay Kit



3.3. LOD and LOQ

The **instrument limit of detection** of the K-AMYLSD assay is 0.007 α -Amylase SD U/g based on the standard assay procedure and a minimum absorbance of 0.02.

The **calculated limit of detection (LOD)** and the **calculated limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the standard procedure of the α -Amylase SD Assay Method (K-AMYLSD).

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as $3 \times \sigma$ of the blank sample solution absorbance and $10 \times \sigma$ of the blank sample solution absorbance, respectively, where σ is the standard deviation of the absorbance values from 10 replicates.
- For α -Amylase SD Assay Kit (K-AMYLSD)

LOD

α -Amylase = 0.003 α -Amylase SD U/g

LOQ

α -Amylase = 0.011 α -Amylase SD U/g

* **Note:** The above detection limits are for samples as used in the assay after any sample preparation, if required. 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 α -amylase Assay Kit (K-AMYLSD) method with a suitable reference value (x_{ref}). For this report, Relative Bias is calculated in per cent as: $b(\%) = x - x_{ref} / x_{ref} \times 100$. The reference material for this purpose is milled wheat with a known α -amylase activity which is supplied with the α -Amylase SD Assay Kit (K-AMYLSD) at 0.1 α -Amylase SD U/g.

Relative Bias $b(\%)$

	n	Ref Material (α -Amylase SD U/g)	Mean (α -Amylase SD U/g)	$b(\%)$
α -Amylase	21	0.1	0.0988	-1.23

3.5. Precision

This report details the reproducibility of the α -Amylase SD Assay Kit (K-AMYLSD), it is a measure of the variability in results on different occasions by different analysts over an extended period of time. In this instance, duplicate analyses of single wheat extracts made on four separate days

Reproducibility

Absorbance (400 nm)											
Sample	DAY 1		DAY 2		DAY 3		DAY 4		MEAN	STDEV	%CV
A	0.133	0.136	0.127	0.128	0.122	0.125	0.121	0.120	0.126	0.006	4.52
B	0.160	0.155	0.156	0.158	0.145	0.140	0.149	0.159	0.152	0.007	4.79
C	0.254	0.239	0.259	0.249	0.258	0.239	0.254	0.249	0.250	0.008	3.18
D	0.407	0.395	0.409	0.413	0.393	0.390	0.410	0.403	0.402	0.009	2.19
E	1.391	1.403	1.409	1.405	1.397	1.385	1.398	1.406	1.399	0.008	0.59

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of α -amylase in various matrices. It is a novel method and is fully automatable for high throughput analysis of samples. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	α -Amylase
Working range (Amylase SD U/g)	0-0.4
LOD (Amylase SD U/g)	0.003
LOQ (Amylase SD U/g)	0.011
Relative Bias <i>b</i> (%)	-1.23
Reproducibility (%CV using flour samples)	≤ 4.79

5. References

Cornaggia, C., Ivory, R., Mangan, D. & McCleary, B. (2016). Novel assay procedures for the measurement of α -amylase in weather-damaged wheat. *Journal of the Science of Food and Agriculture*, 96(2), 404-412.