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Validation Report: β-Amylase Assay Kit (cat. no. K-BETA3)

1. Scope

Megazyme's β-Amylase Assay Kit (K-BETA3) is an enzymatic method used for the rapid measurement and analysis of β-amylase in malt flour, food, beverages and fermentation products. This method was developed in-house and measures β-Amylase in Betamyl-3® U/g. This method has been accepted as a modification of RACI standard method.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the β-Amylase Assay Kit (K-BETA3).

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 β-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 β-Amylase to the sample in the initial extraction steps.

3.2. Working Range

The working range of the β-Amylase Assay Kit (K-BETA3) is up to ~ 0.939 Betamyl-3® U/g based on the standard assay procedure (0.2 mL β-amylase plus
0.1 mL Betamyl-3® Reagent) with an incubation time of 10 min and a maximum absorbance of 1.0.

Following the standard β-Amylase Assay Kit (K-BETA3) assay procedure 0.2 mL pure barley β-amylase was incubated at 40°C for 0-10 min with 0.2 mL of Betamyl-3® Reagent. The reactions were terminated at 2 min intervals and the absorbance values read at 400 nm.

Linearity of the Betamyl-3® Assay
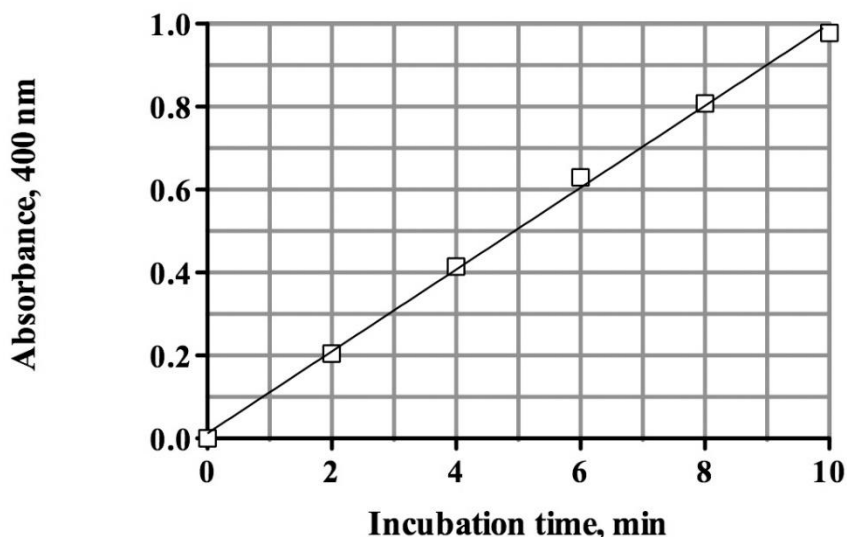
3.3. LOD and LOQ

The **instrument limit of detection** of the K-BETA3 assay is 0.0188 Betamyl-3® 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 whole β-Amylase Assay Kit (K-BETA3) procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as 3 x s'_0; where s'_0 is the standard deviation of a number of samples A1 reading.
- The LOQ is the lowest level at which the kit's performance is acceptably repeatable. LOQ is calculated as k_Q x s'_0; where s'_0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for k_Q is 10.

- For β-Amylase Assay Kit (K-BETA3)

    LOD
    β-Amylase = 0.014 Betamyl-3® U/g

    LOQ
    β-Amylase = 0.046 Betamyl-3® U/g

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* **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.

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the β-Amylase Assay Kit (K-BETA3) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: *b(%) = x – xref / xref x 100*. The reference material for this purpose is malt flour of standardised β-Amylase activity which is supplied with the β-Amylase Assay Kit (K-BETA3) at 22.3 Betamyl-3® U/g.

Relative Bias *b(%)*

	n	Ref Material (Betamyl-3® U/g)	Mean (Betamyl-3® U/g)	*b(%)*
β-Amylase	14	22.3	20.4122	-8.47

3.5. Precision

This report details the reproducibility of the β-Amylase Assay Kit (K-BETA3). It is a measure of the variability in results, across four different days, using four flour samples which were extracted in duplicate with Buffer A or Buffer B and the extracts assayed in duplicate.

Reproducibility

Sample	Absorbance (400 nm)								MEAN	STDEV	%CV
	Day 1		Day 2		Day 3		Day 4				
A	0.685	0.685	0.614	0.618	0.663	0.659	0.695	0.704	0.665	0.034	**5.10**
B	0.759	0.736	0.701	0.712	0.749	0.737	0.773	0.762	0.741	0.025	**3.36**
C	0.644	0.635	0.605	0.595	0.658	0.631	0.752	0.748	0.659	0.060	**9.11**
D	0.848	0.853	0.815	0.783	0.824	0.892	0.853	0.825	0.837	0.032	**3.88**

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of β-Amylase in various matrices. It has been used for many years 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	β-Amylase
Working range (Betamyl-3® U /g)	0.939
LOD (Betamyl-3® U/g)	0.014
LOQ (Betamyl-3® U/g)	0.046
Relative Bias *b*(%)	-8.47
Reproducibility (%CV using flour samples)	≤ 9.11

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