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## Validation Report:  Amylose/Amylopectin Assay Kit, (cat. no. K-AMYL)

### 1. Scope

Megazyme's Amylose/Amylopectin Assay Kit (K-AMYL) is used for the measurement and analysis of amylose/amylopectin ratio and content in cereal starches and flours. It is novel method, based on a Con A precipitation procedure.  Amylose content is given in % (w/w) of total starch.

### 2. Planning

The purpose of this report is to verify and validate the current method as detailed by the Amylose/Amylopectin Assay Kit (K-AMYL).

### 3. Performance characteristics

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

#### 3.1. Selectivity

The assay is based on Concanavalin A (Con A) which, effectively precipitates the amylopectin component of starch (due to its structure), but not the amylose.  The amylopectin can then be removed by centrifugation, leaving the amylose to be easily measured by enzymatic hydrolysis to D-glucose, and subsequent measurement by glucose oxidase/peroxidase reagent.  Total starch is also quantified separately.
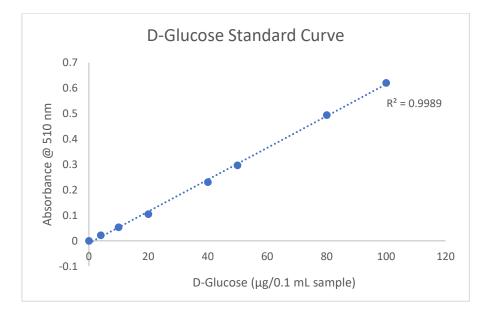
#### 3.2. Working Range

The working range for the Amylose/Amylopectin Assay Kit (K-AMYL) is determined by the D-glucose control provided in the kit.  The glucose measurement (incubation with GOPOD Reagent) is linear between 4 and 100 µg of D-glucose per assay.

0.1 mL of D-glucose standards at various concentrations is incubated with 0.9 mL of sodium acetate buffer and 4.0 mL of GOPOD Reagent for 20 min at 40°C.  The absorbances read against the reagent blank at 510 nm, as specified by the kit data booklet.

The absorbance for 100 µg is ~ 0.7.  If the absorbance of your samples is higher than that of 100 µg of D-glucose control (i.e. higher than 0.7), they must be diluted accordingly.

| D-Glucose Standard Concentration [µg/1.0 mL] | $\Delta A_{510nm}$ |
|---|---|
| 0 | 0.0000 |
| 4 | 0.0219 |
| 10 | 0.0534 |
| 20 | 0.1047 |
| 40 | 0.2308 |
| 50 | 0.2966 |
| 80 | 0.4941 |
| 100 | 0.6203 |

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### 3.3. LOD

If the standard procedure is followed, the smallest differentiating recommended absorbance ΔA is 0.04. The highest ΔA should be lower than the absorbance values obtained for 100 µg of D-glucose.

* **Note:** The above detection limits are for samples as used in the assay, after sample preparations if required (e.g. deproteinisation). 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 Amylose/Amylopectin Assay Kit (K-AMYL) 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 starch supplied with the Amylose/Amylopectin Assay Kit (K-AMYL) with amylose content at 66% w/w total starch.

**Relative Bias *b(%)***

|  | n | Ref Material (%w/w) | Mean (%w/w) | *b*(%) |
|---|---|---|---|---|
| **Amylose** | 12 | 66 | 65.7952 | -0.31 |

### 3.5. Precision

This report details the reproducibility of the Amylose/Amylopectin Assay Kit (K-AMYL), it is a measure of the variability in results, on different days and by different analysts, over an extended period of time.

For the purpose of this report different lot numbers of the kit standard is used as the reference material.

### Reproducibility

|  | n | Ref Material (%w/w) | Mean (%w/w) | Standard Deviation | %CV |
|---|---|---|---|---|---|
| **Amylose** | 12 | 66 | 65.7952 | 0.5625 | 1.29 |

## 4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of amylose 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 | D-Glucose |
|---|---|
| **Working range (µg in assay)** | 4-100 |
| **LOD ($\Delta$A)** | 0.04 |
| **Relative Bias *b*(%) (starch/amylose standard)** | - 0.31 |
| **Reproducibility (%CV using starch/amylose)** | 1.29 |

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