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Validation Report: Pullulanase/Limit-Dextrinase Assay Kit (PullG6 Method) (cat. no. K-PullG6)

1. Scope

Megazyme's Pullulanase/Limit-Dextrinase Assay Kit (PullG6 Method) (K-PullG6) is a colourimetric method used for the rapid measurement and analysis of pullulanase or limit-dextrinase in the starch processing industry. This novel pullulanase/limit-dextrinase method was developed in-house and measures pullulanase/limit-dextrinase as PullG6 Units/mL.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by Pullulanase/Limit-Dextrinase Assay Kit (PullG6 Method) (K-PullG6).

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 absolutely specific for pullulanase/limit-dextrinase.

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 pullulanase/limit-dextrinase to the sample in the initial extraction steps.

3.2. Working Range

The working range of the Pullulanase/Limit-Dextrinase Assay Kit (PullG6 Method) (K-PullG6) is up to ~ 0.177 PullG6 U/mL based on the standard assay procedure (0.1 mL pullulanase/limit-dextrinase plus 0.1 mL PullG6 reagent with an incubation time of 10 min) and a maximum absorbance of 1.0.

The linearity of the PullG6 assay was assessed using Pullulanase M2 (*B. licheniformis*) (**E-PULBL**) at various concentrations.

Following the standard PullG6 assay procedure, 0.1 mL of Pullulanase was incubated at 40°C with 0.1 mL of PullG6 substrate solution. The reactions were terminated at 3 min intervals and the absorbance values read at 400 nm.

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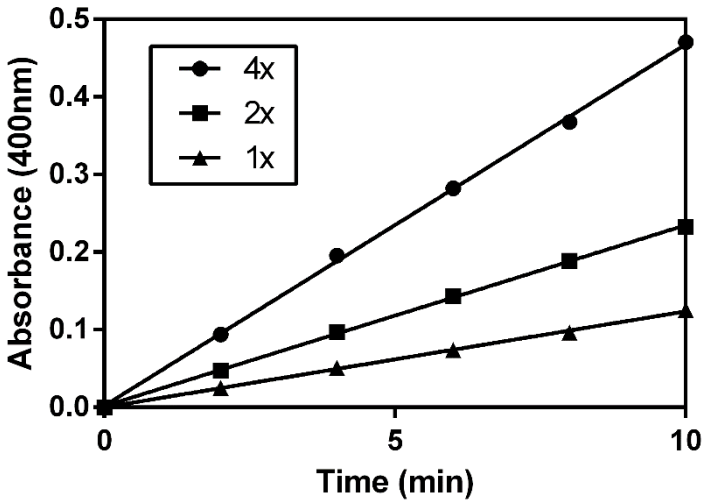
Linearity of the PullG6 Assay



3.3. LOD and LOQ

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 Pullulanase/Limit-Dextrinase Assay Kit (PullG6 Method) (K-PullG6).

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3 x σ of the blank sample solution absorbance and 10 x σ of the blank sample solution absorbance, respectively, where σ is the standard deviation of the absorbance values from 10 replicates.

- For Pullulanase/Limit-Dextrinase Assay Kit (K-PullG6)

  LOD

  Pullulanase/Limit-Dextrinase = 1.2 x 10^{-2} PullG6 U/mL

  LOQ

  Pullulanase/Limit-Dextrinase = 4.0 x 10^{-2} PullG6 U/mL

* **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 Pullulanase/Limit-Dextrinase Assay Kit (K-PullG6) 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 control malt flour of standardised limit-dextrinase activity supplied with the Pullulanase/Limit-Dextrinase Assay Kit (PullG6 Method) (K-PullG6) at 2.1 U/mL.

Relative Bias *b*(%)

	n	Ref Material (U/mL)	Mean (U/mL)	*b*(%)
Limit-Dextrinase	17	2.1	2.1481	2.29

3.5. Precision

This report details the reproducibility of the Pullulanase/Limit-Dextrinase Assay Kit (PullG6 Method) (K-PullG6), it is a measure of the variability in results on different occasions by different analysts over an extended period of time.

Reproducibility

	n	Ref Material (U/mL)	Mean (U/mL)	Standard Deviation	%CV
Limit-Dextrinase	17	2.1	2.1481	0.0741	3.45

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of pullulanase/limit-dextrinase 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	Pullulanase/Limit-Dextrinase
Working range (PullG6 U/mL)	0 - 0.177
LOD (PullG6 U/mL)	1.2×10^{-2}
LOQ (PullG6 U/mL)	4.0×10^{-2}
Relative Bias *b* (%)	2.29
Reproducibility (%CV using kit standard)	≤ 3.45