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Validation Report: Cellulase Assay Kit (CellG5 Method) (cat. no. K-CellG5)

1. Scope

Megazyme's Cellulase Assay Kit (K-CellG5) is a colourimetric method used for the rapid measurement and analysis of cellulase in enzyme preparations and fermentation products. This novel cellulase method was developed in-house and measures cellulase as CellG5 U/mL.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by Cellulase Assay Kit (K-CellG5).

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 cellulase.

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 cellulase to the sample in the initial extraction steps.

3.2. Working Range

The working range of the Cellulase Assay Kit (K-CellG5) is up to ~ 0.176 CellG5 U/mL based on the standard assay procedure (0.1 mL cellulase plus 0.1 mL CellG5 reagent with an incubation time of 10 min) and a maximum absorbance of 1.0.

The linearity of the CellG5 assay was assessed using *Trichoderma longibrachiatum* cellulase (**E-CELTR**) at various concentrations (0-3 U/mL Cellulase). Following the standard CellG5 assay procedure, 0.1 mL of cellulase was incubated at 40°C with 0.1 mL of CellG5 substrate solution. The reactions were terminated at 3 min intervals and the absorbance values read at 400 nm.

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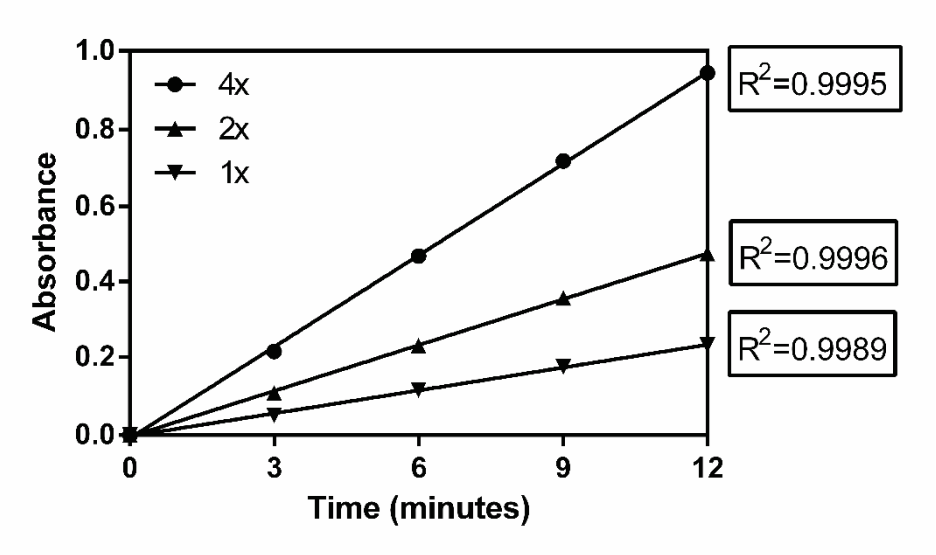
Linearity of the CellG5 Assay



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 Cellulase Assay CellG5 Method (K-CellG5).

- 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 Cellulase Assay Kit (K-CellG5)

  LOD

  Cellulase = 1.2 x 10^{-3} CellG5 U/mL

  LOQ

  Cellulase = 4.1 x 10^{-3} CellG5 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 Cellulase Assay Kit (K-CellG5) 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 cellulase supplied with the Cellulase Assay Kit (K-CellG5) at 3.2 U/mL.

Relative Bias *b*(%)

	n	Ref Material (U/mL)	Mean (U/mL)	*b*(%)
Cellulase	25	3.2	3.0874	-3.52

3.5. Precision

This report details the reproducibility of the Cellulase Assay Kit (K-CellG5), it is a measure of the variability in results on different occasions by different analysts over an extended period of time. In this instance, two analysts performed a series of assays on three different samples ranging in activity from 55-164 CellG5 mU/mL.

Reproducibility

***Trichoderma longibrachiatum* Cellulase**	**55 mU/mL ΔAbs (400 nm)**	**82 mU/mL ΔAbs (400 nm)**	**164 mU/mL ΔAbs (400 nm)**
Experiment 1^A	0.320	0.471	0.925
Experiment 2^A	0.324	0.477	0.925
Experiment 1^B	0.305	0.464	0.910
Experiment 2^B	0.308	0.458	0.883
Standard Dev. (σ)	0.009	0.008	0.020
%CV	2.96	1.75	2.17

Note: A = Analyst 1, B = Analyst 2

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of cellulase 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	Cellulase
Working range (CellG5 U/mL)	0-0.176
LOD (CellG5 U/mL)	1.2×10^{-3}
LOQ (CellG5 U/mL)	4.1×10^{-3}
Relative Bias *b* (%)	-3.52
Reproducibility (%CV using kit standard)	≤ 2.96

5. References

Mangan, D., Cornaggia, C., McKie, V., Kargelis. T. & V. McCleary, B. V. (2016). A novel automatable enzyme-coupled colorimetric assay for *endo*-1,4-β-glucanase (cellulase). *Analytical and Bioanalytical Chemistry*, 408(15), 4159-4168.