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Validation Report: Xylanase Assay Kit (XylX6 Method) (cat. no. K-XylX6)

1. Scope

Megazyme's Xylanase Assay Kit (K-XylX6) is a colorimetric method used for the rapid measurement and analysis of *endo*-1,4-β-xylanase in fermentation broths, industrial enzyme preparations, animal feeds, biofuels and research. This novel xylanase method was developed in-house and measures xylanase as XylX6 Units/mL or Units/g of original enzyme preparation.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by Xylanase Assay Kit (K-XylX6).

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 xylanase.

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 Xylanase to the sample in the initial extraction steps.

3.2. Working Range

The working range of the Xylanase Assay Kit (K-XylX6) is up to ~ 0.176 Xylanase U /mL based on the standard assay procedure (0.05 mL *endo*-xylanase plus 0.05 mL XylX6 Reagent Solution with an incubation time of 10 min) and a maximum absorbance of 1.0.

The linearity of the XylX6 assay was assessed using *Trichoderma longibrachiatum* xylanase (**E-XYTR3**) at various concentrations. Following the standard XylX6 assay procedure, 0.05 mL of Xylanase was incubated at 40°C with 0.05 mL of XylX6 Reagent Solution. The reactions were terminated at 3 min intervals and the absorbance values read at 400 nm.

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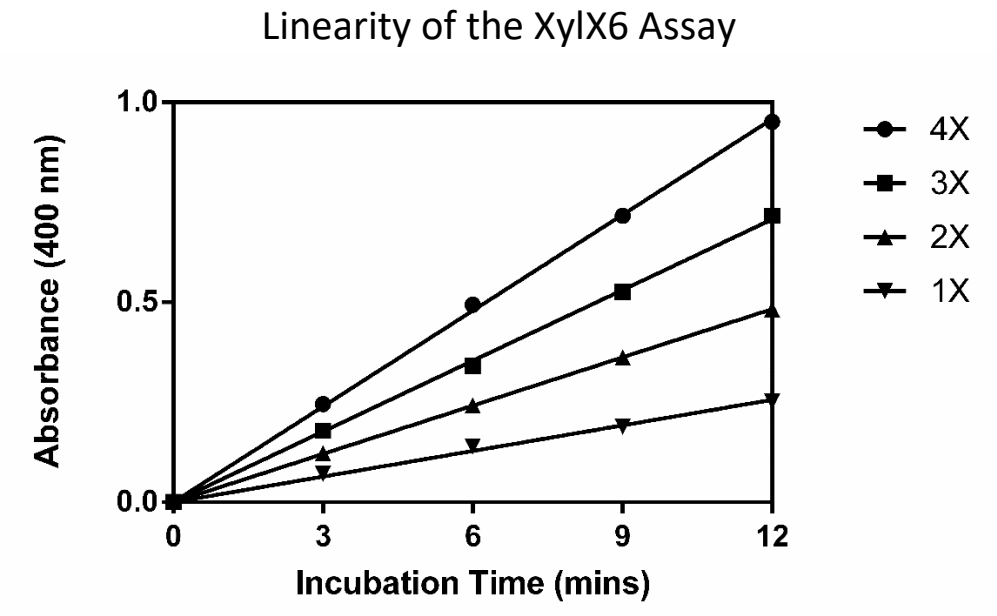

3.3. LOD and LOQ

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 Xylanase Assay XylX6 Method (K-XylX6).

- 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 Xylanase Assay Kit (K-XylX6)

  LOD

  Xylanase = 5.3×10^{-4} XylX6 U/mL

  LOQ

  Xylanase = 1.9×10^{-3} XylX6 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 Xylanase Assay Kit (K-XylX6) 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 Xylanase supplied with the Xylanase Assay Kit (K-XylX6) at 2.85 XylX6 U/mL.

Relative Bias *b*(%)

	n	Ref Material (U/mL)	Mean (U/mL)	*b*(%)
Xylanase	15	2.85	2.8392	-0.38

3.5. Precision

This report details the reproducibility of the Xylanase Assay Kit (K-XylX6), it is a measure of the variability in results on different occasions by different analysts over two consecutive days. In this instance, two analysts performed a series of assays on three different samples ranging in activity from 60-161 XylX6 mU/mL.

Repeatability & Reproducibility

***Trichoderma longibacterium* Xylanase**	**161 mU/mL ΔAbs (400 nm)**	**121 mU/mL ΔAbs (400 nm)**	**60 mU/mL ΔAbs (400 nm)**
Day 1 A(i)	0.982	0.795	0.423
Day 1 A(ii)	0.98	0.794	0.433
Day 2 A(i)	0.993	0.807	0.437
Day 2 A(ii)	0.993	0.811	0.438
Day 1 B(i)	1.016	0.79	0.413
Day 1 B(ii)	1.028	0.764	0.413
Day 2 B(i)	1.055	0.784	0.419
Day 2 B(ii)	1.04	0.803	0.413
Standard Dev. (σ)	**0.028**	**0.015**	**0.011**
% CV	**2.8**	**1.9**	**2.6**

Note*: A = Analyst 1, B = Analyst 2, (i) = Extract 1, (ii)= Extract 2*

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of xylanase 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	Xylanase
Working range (XylX6 U/mL)	0 - 0.176
LOD (XylX6 U/mL)	5.3×10^{-4}
LOQ (XylX6 U/mL)	1.9×10^{-3}
Relative Bias *b* (%)	-0.38
Reproducibility (%CV)	≤ 2.8

5. References

Mangan, D., Cornaggia, C., Liadova, A., Draga, A., Ivory, R., Evans, D. & McCleary, B. (2018). Development of an automatable method for the measurement of *endo*-1,4-β-xylanase activity in barley malt and initial investigation into the relationship between *endo*-1,4-β-xylanase activity and wort viscosity. *Journal of Cereal Science*, 84, 90-94.