Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

Validation Report: D-Xylose Assay Kit (cat. no. K-XYLOSE)

1. Scope

Megazyme's D-Xylose Assay Kit (K-XYLOSE) is an enzymatic method used for the rapid measurement and analysis of xylose in plant extracts, culture media/supernatants and other materials. This novel method was developed in-house and measures xylose in g/L.

2. Planning

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

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

All reported forms of β-xylose dehydrogenase also act on D-glucose. At concentrations of D-glucose similar to the levels of D-xylose measured in this assay, the current enzyme acts slowly. However, at high concentrations of D-glucose, the rate of reaction is very significant and thus problematic. In many instances, there will be a need to measure D-xylose in the presence of high concentrations of D-glucose (e.g. in mixtures of sugars obtained on acid hydrolysis of wheat flour), thus it is essential to remove the D-glucose. In the current assay protocol, this is achieved by a short pre-incubation of the sample extract with hexokinase in the presence of an excess of ATP.

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 xylose to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the D-Xylose Assay Kit (K-XYLOSE) standard procedure. 0.1mL of xylose standard was used as sample, with a range of concentrations (0.02-1 g/L) which corresponds to 2-100 µg of D-xylose per cuvette. Absorbance A2 was read after 6 min, at 340 nm and at 25°C as recommended in the procedure.

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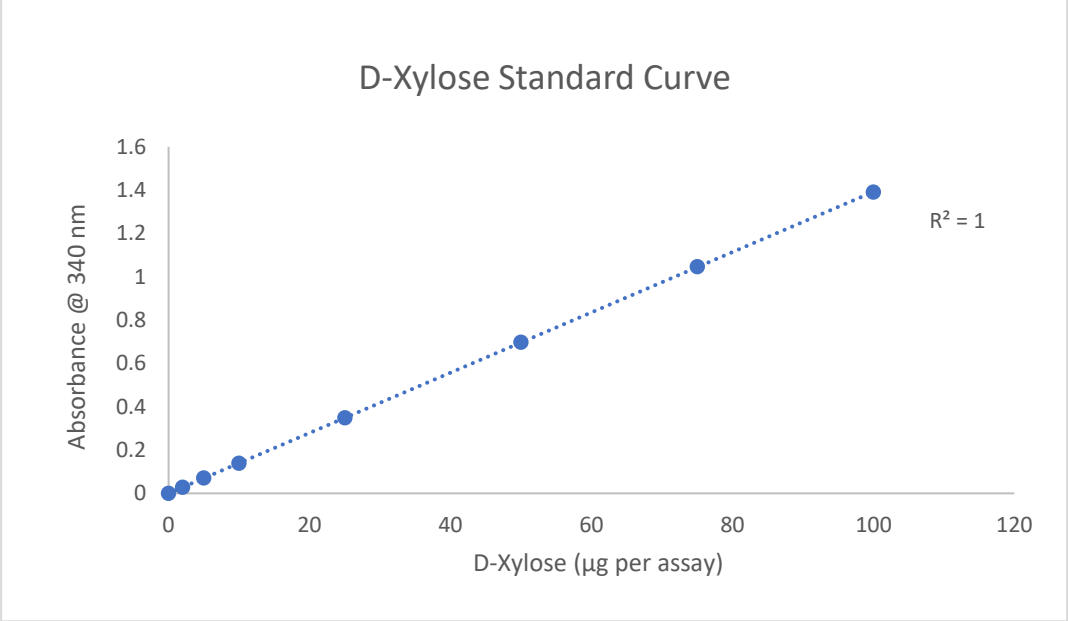
Xylose Concentration [µg/assay]	ΔA_{340nm}
0	0.000
2	0.028
5	0.070
10	0.139
25	0.349
50	0.697
75	1.046
100	1.390



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3.3 LOD and LOQ

The **instrument limit of detection,** as per kit booklet, is 0.701 mg/L which is derived from an absorbance difference of 0.020 and the maximum sample volume of 2.00 mL.

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 D-Xylose Assay Kit (K-XYLOSE) measurement 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 kQ x s'0; where s'0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10

- For D-Xylose Assay Kit (K-XYLOSE)

  LOD – For 2.0mL of sample (maximum volume)
  D-Xylose = 0.140 mg/L

  LOQ – For 2.0mL of sample (maximum volume)
  D-Xylose = 0.495 mg/L

* **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.

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3.4 Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with D-Xylose Assay Kit (K-XYLOSE) 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 D-xylose supplied with the D-Xylose Assay Kit (K-XYLOSE) at 0.25 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Xylose	17	0.25	0.2504	0.17

3.5 Precision

This report details the reproducibility of the D-Xylose Assay Kit (K-XYLOSE), 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 (g/L)	Mean (g/L)	Standard Deviation	%CV
D-Xylose	17	0.25	0.2504	0.003	1.14

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of Xylose 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-Xylose
Working range (µg in cuvette)	2-100
LOD (mg/L)	0.140
LOQ (mg/L)	0.495
Relative Bias *b*(%)	0.17
Reproducibility (%CV using D-xylose)	1.14