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Validation Report: D-Glucuronic/D-Galacturonic Acid Assay Kit (cat. no. K-URONIC)

1. Scope

Megazyme's D-Glucuronic/D-Galacturonic Assay Kit is a simple, reliable and accurate method for the measurement and analysis of D-hexuronic acids (specifically D-glucuronic acid and D-galacturonic acid) in plant extracts, culture media/supernatants and other materials. This method was developed in-house and measures D-Glucuronic or D-Galacturonic Acid in g/L.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by D-Glucuronic/D-Galacturonic Acid Assay Kit (K-URONIC).

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

The assay is specific for D-glucuronic acid (D-glucuronate) and D-galacturonic acid (D-galacturonate). This assay kit does not differentiate between these uronic acids.

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 D-Glucuronic/D-Galacturonic Acid to the sample in the initial extraction steps.

3.2. Working Range

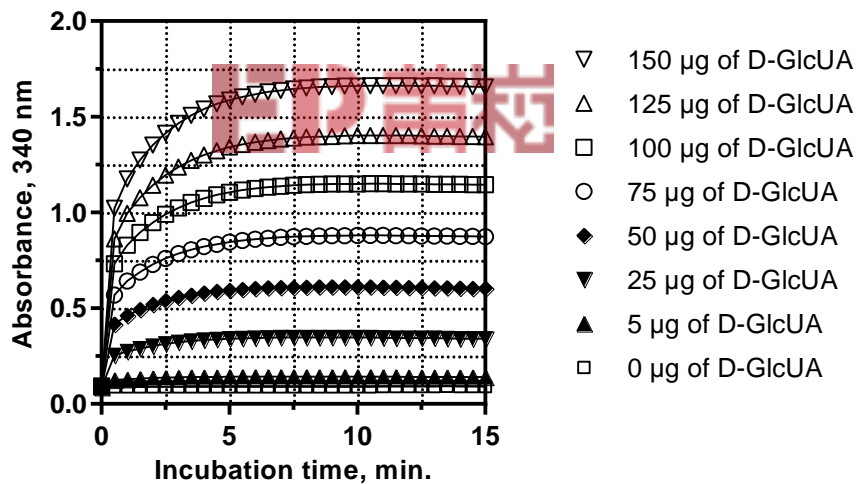
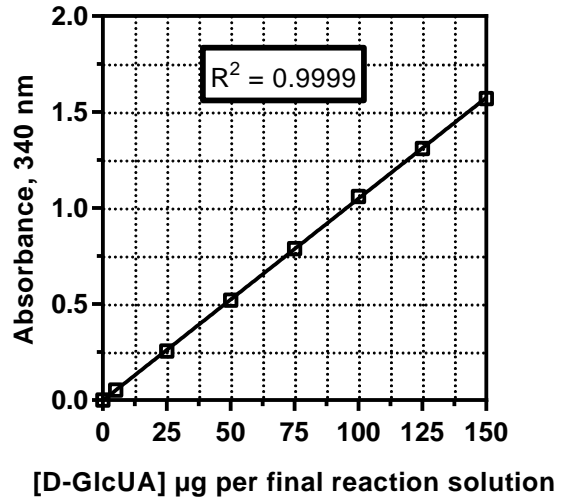
Assay follows the D-Glucuronic/D-Galacturonic Acid Assay Kit (K-URONIC) standard procedure. D-glucuronic acid standard was used as a sample across a range of concentrations (0.05-1.5 g/L D-glucuronic Acid) which corresponds to 5-150 µg of D-glucuronic acid per cuvette.

The working range is linear between 5-150 µg of D-glucuronic acid or D-galacturonic acid per assay.



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Glucuronic Acid Concentration [µg/cuvette]	ΔA_{340nm}
0	0.0000
5	0.0518
25	0.2566
50	0.5216
75	0.7893
100	1.0600
125	1.3111
150	1.5710



3.3. LOD and LOQ Range

The **instrument limit of detection** for the standard manual assay procedure is 0.777 mg/L, which is derived from an absorbance difference of 0.02 with 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-Glucuronic/D-Galacturonic Acid Assay Kit (K-URONIC) measurement procedure.



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- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as $3 \times s'_0$; 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 $k_Q \times s'_0$; where s'_0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for k_Q is 10.
- For D-Glucuronic/D-Galacturonic Acid Assay Kit (K-URONIC)

LOD – For 2.0 mL of sample (maximum volume)

D-Glucuronic/D-Galacturonic Acid = 0.004 mg/L

LOQ – For 2.0 mL of sample (maximum volume)

D-Glucuronic/D-Galacturonic Acid = 0.311 mg/L

* **Note:** The above detection limits are for samples as used in the assay after sample preparation, 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 D-Glucuronic/D-Galacturonic Acid Assay Kit (K-URONIC) method with a suitable reference value (x_{ref}). For this report, Relative Bias is calculated in per cent as: $b(\%) = \frac{x - x_{ref}}{x_{ref}} \times 100$. The reference material for this purpose is D-glucuronic acid supplied with the D-Glucuronic/D-Galacturonic Acid Assay Kit (K-URONIC) at 0.5 g/L.

Relative Bias $b(\%)$

	n	Ref Material (g/L)	Mean (g/L)	$b(\%)$
D-Glucuronic Acid	22	0.5	0.4990	- 0.19



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3.5. Precision

This report details the reproducibility of the D-Glucuronic/D-Galacturonic Acid Assay Kit (K-URONIC), 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-Glucuronic Acid	22	0.5	0.4990	0.0073	1.46

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-Glucuronic or D-Galacturonic Acid 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-Glucuronic Acid
Working range (μg in cuvette)	5 - 150
LOD (mg/L)	0.004
LOQ (mg/L)	0.311
Relative Bias <i>b</i> (%)	- 0.19
Reproducibility (%CV using kit standard)	1.46