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Validation Report: Lactulose Assay Kit (cat. no. K-LACTUL)

1. Scope

Megazyme's Lactulose Assay Kit (K-LACTUL) is an enzymatic method that was developed at Megazyme and is suitable for the specific, rapid and sensitive measurement and analysis of lactulose in milk and milk-based samples. Reagents included in this kit may also be prepared for use in the procedure described by ISO Method 11285:2004 and NY/T 939-2016 (modified).

2. Planning

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

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 lactulose.

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 ammonia to the sample in the initial extraction steps.

3.2. Working Range

The assay follows the Lactulose Assay Kit (K-LACTUL) standard procedure.

Samples (1 mL) of pure lactulose were used at concentrations (0.16-0.5 mg/mL) which corresponds to 2-65 µg of lactulose per cuvette.

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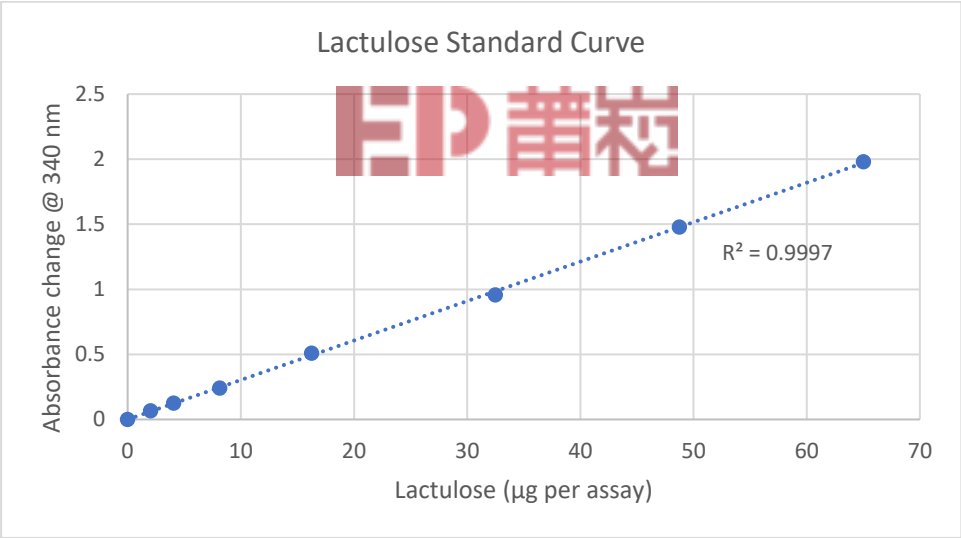
Lactulose Concentration [µg/cuvette]	ΔA_{340nm}
0	0.000
2.0	0.066
4.1	0.125
8.1	0.241
16.3	0.51
32.5	0.956
48.8	1.479
65.0	1.981



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 4.8 mg/L, which is derived from an absorbance difference of 0.02 with a sample volume of 1.00 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 Lactulose Assay Kit (K-LACTUL) 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 k_Q x 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 Lactulose Assay Kit (K-LACTUL)

  LOD – For 1.0 mL of sample (maximum volume)
  Lactulose = 5.3 mg/L

  LOQ – For 1.0 mL of sample (maximum volume)
  Lactulose = 17.6 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. LOD and LOQ Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with Lactulose Assay Kit (K-LACTUL) 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 lactulose supplied with the Lactulose Assay Kit (K-LACTUL) at 0.1 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
Lactulose	14	0.1	0.0983	-1.75

3.5. Precision

This report details the repeatability and reproducibility of the Lactulose Assay Kit (K-LACTUL). Repeatability is a measure of the variability in results when a measurement is performed by a single analyst using the same equipment over a short timescale. Reproducibility is a measure of the variability in results when a measurement is performed by different analysts using different equipment over an extended timescale.

For the purpose of this report different lot numbers of the kit standard is used as the reference material.

Repeatability

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
Lactulose	6	0.1	0.0972	0.0036	4.22

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
Lactulose	14	0.1	0.0983	0.0036	3.65

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4. Performance characteristics

The method outlined in this document is a robust, quick and easy method for the measurement of lactulose 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	Lactulose
Working range (µg in cuvette)	2-65
LOD (mg/L)	5.3
LOQ (mg/L)	17.6
Relative Bias *b* (%)	-1.75
Repeatability (%CV using kit standard)	4.22
Reproducibility (%CV using kit standard)	3.65