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## **Validation Report: Lactose/Galactose Assay Kit (Rapid) (cat. no. K-LACGAR)**

### **1. Scope**

Megazyme's Lactose/Galactose Assay Kit (K-LACGAR) is used for the rapid test of lactose, D-galactose and L-arabinose in food and plant products. Galactose dehydrogenase can be used the measurement and analysis of both D-galactose and L-arabinose. This kit is suitable for the analysis of lactose in "low-lactose" or "lactose-free" samples which contain high levels of monosaccharides. Methods based on this principle have been accepted by AOAC 2006.06, NBN, DIN, GOST and IDF.

### **2. Planning**

The purpose of this report is to verify and validate the current method as detailed by Lactose/Galactose Assay Kit (K-LACGAR).

### **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 lactose and D-galactose.

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 lactose to the sample in the initial extraction steps.

#### **3.2. Working Range**

Assay follows the Lactose/Galactose Assay Kit (K-LACGAR) standard procedure. 0.2 mL of D-galactose standard was used as sample, with a range of concentrations (0.02-0.4 g/L D-galactose) which corresponds to 4-80 µg of D-galactose per cuvette. Absorbance A<sub>2</sub> was read after 5 min, at 340 nm and at 25°C as recommended in the procedure.

Linearity of lactose samples was also assessed. 0.2 mL of a lactose standard was used as sample, with a range of concentrations (0.04-0.8 g/L lactose) which corresponds to 8-160 µg of lactose per cuvette. 0.20 mL of lactose sample was treated with 0.20 mL of β-galactosidase as per assay procedure, and then

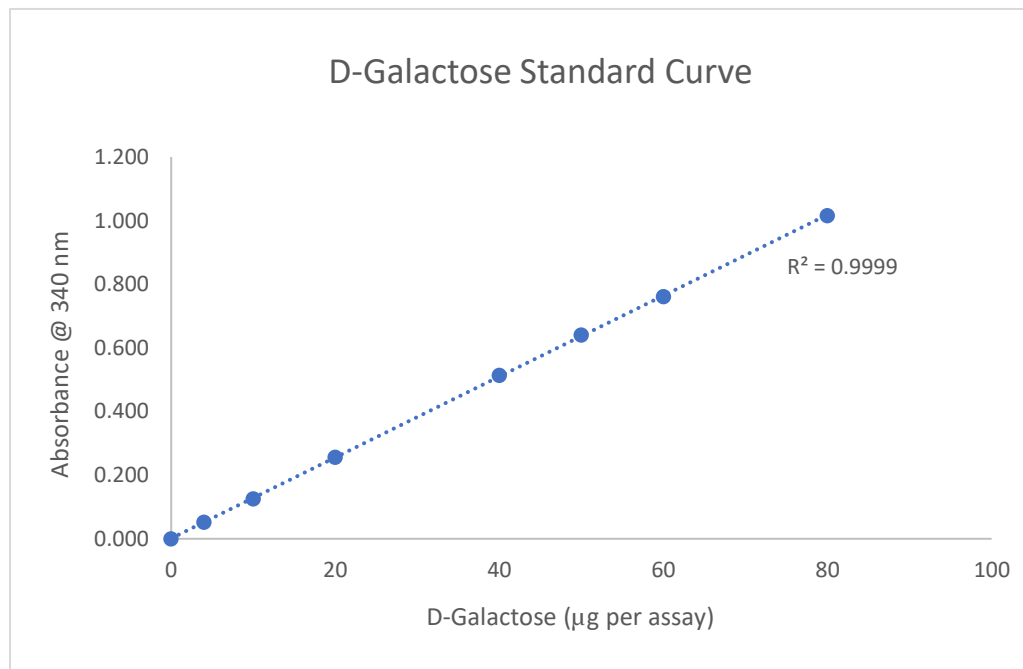


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proceeded to measure as per galactose procedure. Absorbance A2 was read after 5 min, at 340 nm and at 25°C as recommended in the procedure. This result gives the absorbances for lactose + D-galactose in the sample. Lactose can then be determined from the difference in absorbances between (Lactose + D-Galactose) – D-Galactose.

D-Galactose Concentration [ $\mu\text{g}/\text{assay}$ ]	$\Delta A_{340 \text{ nm}}$
0	0.000
4	0.052
10	0.126
20	0.257
40	0.514
50	0.641
60	0.762
80	1.016

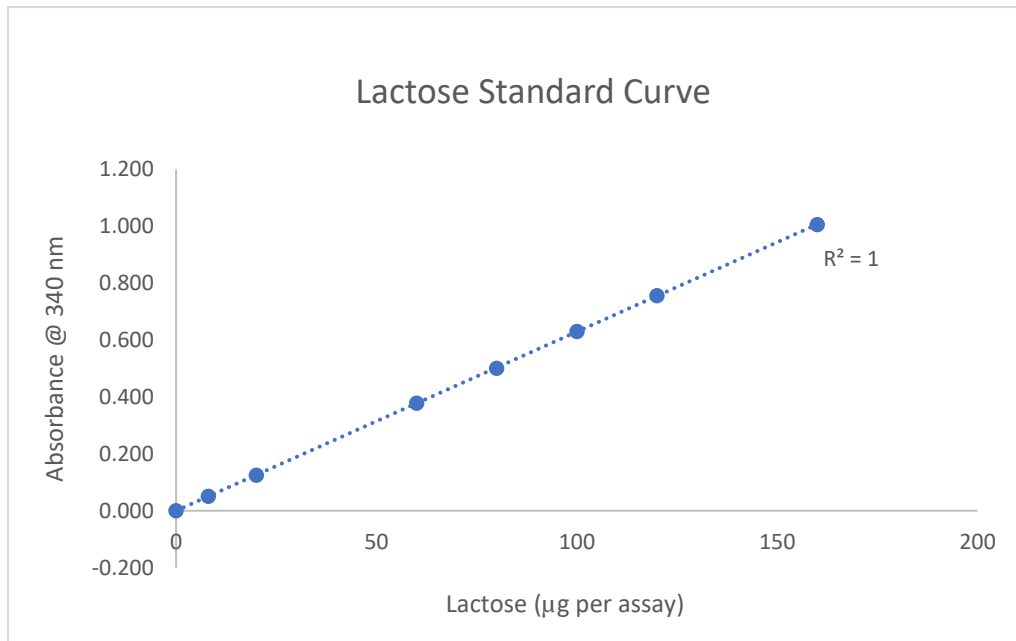




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Lactose Concentration [ $\mu\text{g}/\text{assay}$ ]	$\Delta A_{340 \text{ nm}}$
0	0.000
8	0.051
20	0.125
60	0.378
80	0.501
100	0.630
120	0.756
160	1.006





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**3.3. LOD and LOQ**

The **instrument limit of detection**, as per kit booklet, is 1.48 mg of D-galactose/L, which is derived from an absorbance difference of 0.020 with the maximum 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 Lactose/Galactose Assay Kit (K-LACGAR) procedure.

- 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  $kQ \times s'0$ ; where  $s'0$  is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10.
- For Lactose/Galactose Assay Kit (K-LACGAR)

**LOD – For 1.0 mL of sample (maximum volume)**

D-Galactose = 0.24 mg/L (equates to 0.456 mg/L lactose)

**LOQ – For 1.0 mL of sample (maximum volume)**

D-Galactose = 0.700 mg/L (equates to 1.33 mg/L lactose)

\* **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 the Lactose/Galactose Assay Kit (K-LACGAR) method with a suitable reference value ( $x_{ref}$ ). For this report, Relative Bias is calculated in per cent as:  $b(\%) = x - x_{ref} / x_{ref} \times 100$ . The reference material for this purpose is D-galactose supplied with the Lactose/Galactose Assay Kit (K-LACGAR) at 0.4 g/L.

#### Relative Bias $b(\%)$

	n	Ref Material (g/L)	Mean (g/L)	$b(\%)$
D-Galactose	24	0.4	0.4037	0.92

### 3.5. Precision

This report details the reproducibility of the Lactose/Galactose Assay Kit (K-LACGAR), 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 are used as the reference material.

#### Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
D-Galactose	24	0.4	0.4037	0.0027	0.68



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Repeatability of this kit has been assessed using various low lactose dairy samples. Assay followed procedure “B” (For “low-lactose” or “lactose-free” samples containing high levels of monosaccharides).

This is a measure of the variability in results by a single analyst, using real samples, using the same equipment and over a short period of time. The use of dairy samples shows one of the many applications of this kit.

### Repeatability

	n	Mean (% w/w or w/v)	Standard Deviation	%CV
<b>Milk, lactose</b>	4	0.025	0.001	3.87
<b>Cream, lactose</b>	4	0.094	0.0017	1.81
<b>Cream cheese, lactose</b>	4	0.038	0.0021	5.39

### Recovery

Recovery experiments were also performed by spiking samples with a known amount of lactose before performing all sample pre-treatment steps.

	Sample Only (% w/w or w/v)	Spiked Sample (% w/w or w/v)	Recovery %
<b>Milk</b>	0.025	0.158	99.3
<b>Cream</b>	0.094	0.228	100.0
<b>Cream cheese</b>	0.038	0.178	104.5
<b>Lactose</b>	0.134	-	-



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**4. Conclusion**

The method outlined in this document is a robust, quick and easy method for the measurement of lactose/D-galactose 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.

<b>Validation Summary</b>	<b>D-Galactose</b>
<b>Working range (<math>\mu\text{g}</math> in cuvette)</b>	4-80
<b>LOD (mg/L)</b>	0.24
<b>LOQ (mg/L)</b>	0.7
<b>Relative Bias <i>b</i>(%)</b>	0.92
<b>Reproducibility (%CV using D-galactose)</b>	0.68
<b>Repeatability (%CV milk, lactose)</b>	3.87
<b>Repeatability (%CV cheese, lactose)</b>	1.81
<b>Repeatability (%CV Cream cheese, lactose)</b>	5.39