

**D-FRUCTOSE / D-GLUCOSE
(LIQUID READY™)
VALIDATION REPORT**

**SKU: 700007621
K-FGLQR**

11/24



INTRODUCTION:

The D-Fructose / D-Glucose Assay Kit (Liquid Ready™) is a robust, quick and easy method for the measurement of D-Glucose and D-Fructose in various matrices and is fully automatable for high throughput analysis of samples. Data presented in this report validates that this method is fit for the purpose intended.

RECOMMENDATIONS FOR ANALYSIS:

Please reach out to your local sales representative or to the technical team should you require any assistance, particularly in relation to assay troubleshooting, data analysis, additional matrix testing and application support in relation to automated analyzers.

- This test should only be carried out by trained laboratory personnel. The product instructions must be followed to ensure an accurate and robust result.
- Store the kit at 2 - 8 °C.
- Ensure all kit components come to room temperature 20 - 25 °C before use.
- Use the contents of bottles 1, 2 & 3 as supplied.
- Use a repetitive pipettor to add reagents to the cuvette for increased accuracy.
- The reagent blank value must be determined once for each run and subtracted from each sample result.
- Users should perform matrix validation experiments prior to routine use. This process will highlight any problematic matrices encountered.
- The Carrez Clarification kit (K-CARREZ; 700004270) is recommended for fat removal and deproteinization if necessary.
- Use separate tips for each sample extract and control solution to avoid cross-contamination. Additionally, pre-flush the tip before pipetting.
- When testing solid samples, ensure a representative portion is taken and homogenized before weighing.

EQUIPMENT (RECOMMENDED):

1. Syringe filters (e.g. AGILENT™ Nylon or equivalent, 0.2 micron); alternatively, eppendorf tubes and centrifuge.
2. Sartorius™ grade 292 filter paper or equivalent.
3. Blender (e.g. Nutribullet™).
4. Analytical balance and spatulas.
5. Wide-mouth bottles (e.g. Duran® bottles, 100 mL and 1 L).
6. Glass graduated cylinders (25 mL, 50 mL) and volumetric flasks (100 mL).
7. pH-meter or equivalent.
8. Refrigerator or ice bath (or equivalent).
9. Stir plate.

SUMMARY OF PERFORMANCE DATA:

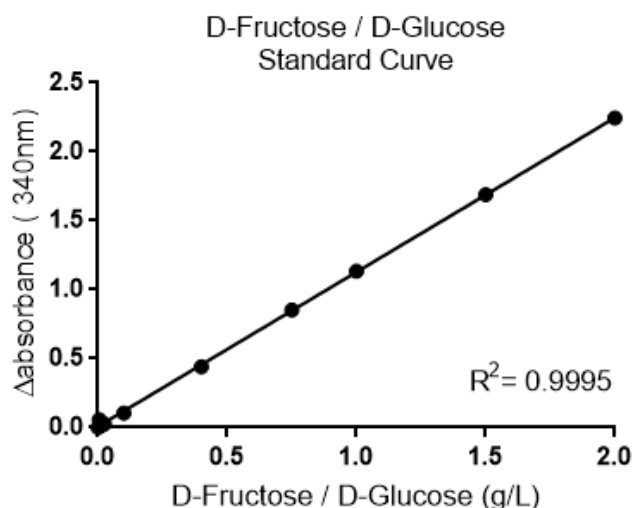
All testing was performed using the standard manual assay described in the product instructions document. Results are summarised in the table below:

Recommended working range	0.025 g/L – 1.500 g/L
Limit of Detection (LOD)	0.006 g/L
Limit of Quantification (LOQ)	0.016 g/L
Limit of Precision	0.025 g/L
Specificity	D-Fructose / D-Glucose can be measured as total sugars or measured separately.
Bias %	-1.84 % to 3.40%
Acceptable Recovery of Standards	95% - 105%
Stability	24 months shelf life from date of manufacture, see product label for expiry
	Kit performance maintained after 3 freeze-thaw cycles
Robustness	< 15 mins (Total sugars) < 23 mins (D-Fructose / D-Glucose) to reach completion at 20°C, 25°C and 37°C
Repeatability	CV < 5% for pure D-Fructose / D-Glucose samples
	CV < 10% for a range of matrices tested
Selectivity/Cross-reactivity	No cross reactivity observed with interfering agents tested
Matrix Interference	Recovery between 95% - 105% in red wine, white wine, apple juice, beer, orange juice, sour jellies, fruit liquid yogurt, grapes and breakfast cereal.

LINEARITY AND WORKING RANGE:

The recommended linear measurement range is from 0.025 g/L to 1.5 g/L of D-Fructose / D-Glucose. Samples with concentrations up to 2.0 g/L can be analyzed without dilution, however assay repeatability/robustness may be affected at concentrations above 1.5 g/L. Samples containing higher levels of analyte can be diluted with distilled water before measurement. Values below 0.01 g/L will not fall within the acceptance criteria.

D-Fructose / D-Glucose (g/L)	$\Delta_{\text{Absorbance}}$ (340 nm)	Recovery (%)
0	0	0
0.01	0.011	93.9
0.025	0.027	96.4
0.005	0.057	101.1
0.100	0.106	101.1
0.400	0.440	97.5
0.750	0.854	100.9
1.000	1.135	100.6
1.500	1.689	99.8
2.000	2.244	99.5



LIMIT OF DETECTION, QUANTIFICATION AND PRECISION:

The LOD is the lowest concentration of the analyte that can be detected by the method. This was determined by testing 20 replicates of the blank (i.e. adding 100 μL of water instead of sample). The $\Delta A_{\text{Limit of Detection}}$ is calculated as $3.3 \times s'0$; where $s'0$ is the standard deviation of a number of samples $\Delta_{\text{Absorbance}}$ reading.

The LOQ is the lowest level at which the kit's performance is acceptably repeatable. This was determined by testing 20 replicates of the blank (i.e. adding 100 μL of water instead of sample). The $\Delta A_{\text{Limit of Quantification}}$ is calculated as $kQ \times s'0$; where $s'0$ is the standard deviation of a number of samples $\Delta_{\text{Absorbance}}$ reading. The IUPAC default value for kQ is 10.

The Limit of Precision (g/L) is the analyte concentration at which it was experimentally determined that acceptable recoveries ($\pm 5\%$) are routinely achieved. It was assessed by measuring 12 replicates of the lowest analyte concentrations, starting at LOQ level.

$\Delta A_{\text{Limit of Detection}}$	Limit of Detection (g/L)	$\Delta A_{\text{Limit of Quantification}}$	Limit of Quantification (g/L)	Precision Limit (g/L)
0.006	0.0053	0.0182	0.0162	0.025

Note: The above detection limits were calculated based on assay concentration (i.e. samples post-extraction). The dilution used in pre-treatment must be accounted for when establishing the detection limits for specific samples.

TRUENESS AND BIAS:

The Trueness was tested using validated aqueous D-Fructose / D-Glucose standards. The *bias* is the comparison of the mean of the results (X) achieved using the standard manual protocol with a suitable reference material.

- Relative Bias is calculated in percent as: $b(\%) = (X - X_{ref}) / X_{ref} \times 100$

Reference material (g/L)	Replicates, n	% CV	% Recovery	% Bias
0.025	12	2.607	103.39	3.395
0.750	12	0.535	100.53	0.533
1.500	12	0.011	98.160	-1.840

Recovery of sample solutions was within the acceptance criteria of $100 \pm 5\%$ and %CV below 5%. The *bias* for this method ranges from -1.84% to 3.40%.

INTERFERENCE AND SELECTIVITY:

Selectivity

The assessment of the selectivity of this method in the presence of interfering compounds was tested by spiking a set concentration of interfering agent with a known concentration of validated aqueous D-Fructose / D-Glucose standard and assessing the recovery. Concentrations of interfering agent were selected based on what were considered to be levels likely to be found in relevant samples.

NOTE: Interfering substances in the sample being analyzed 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-Fructose / D-Glucose to the sample in the initial extraction steps.

Compound tested	Concentration of interferent in assay (g/L)	Recovery of D-Fructose / D-Glucose (%)
D-Galactose	10	99.4
D-Mannose	5	100.5
Sucrose	200	98.9
Lactose	20	100.4
Glycerol	10	99.4
Sorbitol	200	99.7
L-Malic acid	10	99.4
L-Tartaric acid	10	99.4
Citric acid	5	99.7
Maleic Acid	5	99.6
Succinic Acid	5	99.2
Galacturonic acid	10	98.8
D/L-Lactic acid	20	98.7
Acetic acid	50	97.9
Ascorbic Acid	50	97.4
Sodium Chloride	20	100.3
Calcium Chloride	100	98.8
Ethanol	120	99.3

All recoveries were within the acceptance criteria of $100 \pm 5\%$. No cross-reactivity was found in any of the tested substances.

Matrix Interference

The assessment of the interference of this method when using specific complex matrices was tested by spiking extracted samples with a known concentration of validated aqueous ammonia standard and assessing the recovery.

NOTE: All matrices were prepared according to the sample preparation methods described in the product instruction document which can be found on the product webpage.

Matrix tested	Replicates, n	D-Glucose Spike Recovery (%)	D-Fructose Spike Recovery (%)
Red Wine	2	102.28	102.52
White Wine	2	100.29	100.01
Apple Juice	2	100.79	98.62
Orange Juice	2	98.97	99.29
Breakfast cereal	2	100.03	100.78
Sour jellies	2	104.96	101.57
Fruit Yogurt	2	100.47	99.48
Grapes	2	99.50	98.74

Eight matrices were tested; all samples fall within 95-105% recovery. Furthermore, the average %CV for all spike recoveries was 2.25% for D-Glucose and 1.95% for D-Fructose which demonstrates the precision and specificity of this method.

ROBUSTNESS AND STABILITY:

Storage Temperature

To assess the storage stability of the test kit components, all kit components were stored at 4°C. Real time monthly performance and enzyme activity testing was conducted, and where relevant slope predictions were used to estimate the product shelf life.

Storage Temperature	Reagent Tested	Stability Data
4°C	Reagent 1	24 months shelf life from date of manufacture, see product label for expiry
	Reagent 2	
	Reagent 3	

The storage robustness of this kit was also tested by performing three freeze-thaw cycles. All kit components were frozen at -20°C overnight and allowed to thaw before testing. This freeze-thaw cycle was repeated 3 times using the same components.

Total Sugars Recovery (g/L)				
Expected D-Fructose / D-Glucose (g/L)	T0	1st Cycle	2nd Cycle	3rd Cycle
0.025	0.025	0.026	0.025	0.025
0.750	0.775	0.772	0.757	0.758
1.500	1.513	1.492	1.482	1.490

Recovery of sample solutions was within the acceptance criteria of $100 \pm 5\%$. ANOVA evaluation of the freeze-thaw cycles shows that there is no significant difference in assay performance over three consecutive freezing cycles of K-FGLQR reagents.

Assay Temperature

Enzymatic test kits are known to be subject to variation when tested under different environmental conditions; temperature being a key parameter that influences reaction rate and analyte recovery. The D-Fructose / D-Glucose Assay kit (Liquid Ready™) was tested using different reaction incubation temperatures (20°C, 25°C and 37°C); recoveries and reaction times were analyzed.

	Total Sugars Recovery (g/L)		
Expected D-Fructose / D-Glucose (g/L)	20°C	25°C	37°C
0.025	0.025	0.025	0.025
0.75	0.761	0.775	0.758
1.50	1.486	1.513	1.481
Reaction Time	15 min	15 min	10 min
<i>Recommended time</i>	<i>15 minute reaction time is recommended</i>		

ANOVA evaluation of the effect of temperature in assay shows that there is no significant difference in recovery at the temperatures tested. All recoveries were shown to be within acceptance criteria of $100 \pm 5\%$ with a faster reaction time observed at 37°C. The recommended reaction time for total sugars for K-FGLQR is 15 minutes.

	D-Glucose		
1st reaction	20°C	25°C	37°C
Reaction Time	5 mins	4 mins	2 mins
2nd reaction	D-Fructose		
Reaction Time	15 mins	15 mins	7 mins

PRECISION AND REPEATABILITY:

Precision

Precision is a measure of the variability in results, on different days and by different analysts, over a period of time and using different lots of the test kit. The precision of the D-Fructose / D-Glucose Assay kit (Liquid Ready™), was investigated using validated aqueous D-Fructose / D-Glucose samples.

D-Fructose / D-Glucose (g/L)	Replicates, n	Mean (g/L)	Standard Deviation	%CV
0.010	24	0.010	0.0003	2.693
0.750	24	0.749	0.0032	0.433
1.500	24	1.496	0.006	0.398

All recoveries were shown to be within acceptance criteria of $100 \pm 5\%$ and %CV below 5%. This demonstrates that the method is precise and repeatable when using validated aqueous D-Glucose / D-Fructose standards.

NOTE: Users should perform matrix validation work prior to routine use. This process will highlight any problematic matrices encountered. If you have questions about these or other matrices, please contact your local sales representative for support.

Matrix Repeatability

Matrix repeatability was tested by one analyst over three days using a range of selected matrices. Sample preparation was performed on a daily basis to assess precision of analyte extraction as well as precision of the test method. All samples were prepared following the matrix-specific method stated in the product instructions.

Matrix	Replicates, n	Mean result (g/L or g/100g)	Standard Deviation	%CV
Red wine	6	0.354	0.031	8.750
White wine	6	0.820	0.015	1.883
Apple juice	6	20.46	1.355	6.619
Breakfast Cereal	6	0.614	0.007	1.081
Orange Juice	6	18.73	0.533	2.847
Sour Jellies	6	11.60	0.357	3.074
Fruit Yogurt	6	1.537	0.075	4.863
Grapes	6	7.654	0.364	4.756

All sample analysis resulted in %CV values of <10%. This demonstrates that the method is precise and repeatable when testing complex matrices.

METHOD AUTOMATION:

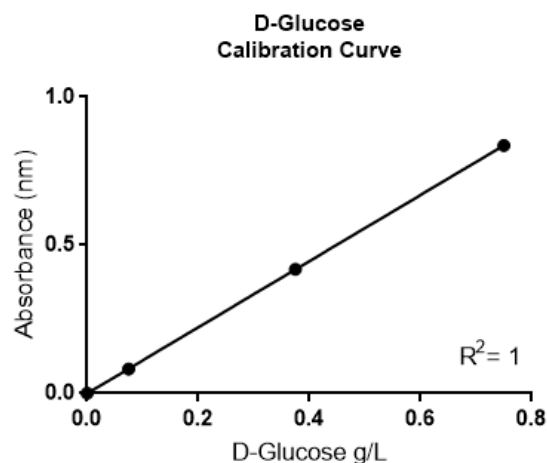
The D-Fructose / D-Glucose Liquid Ready™ kit was designed for biochemistry analyzers. It is easily adapted to any instrument.

D-Glucose and D-Fructose (Total) content can be determined in a single test however separate quantification of D-Glucose and D-Fructose should be performed in two assays as described below, where D-Fructose content is the difference between the result for D-Glucose and D-Glucose/D-Fructose (Total) using a cubic spline fit.

NOTE: For each batch of samples that is applied to the determination of D-Glucose and D-Glucose/D-Fructose (Total) a calibration curve must be performed concurrently using the same batch of reagents.

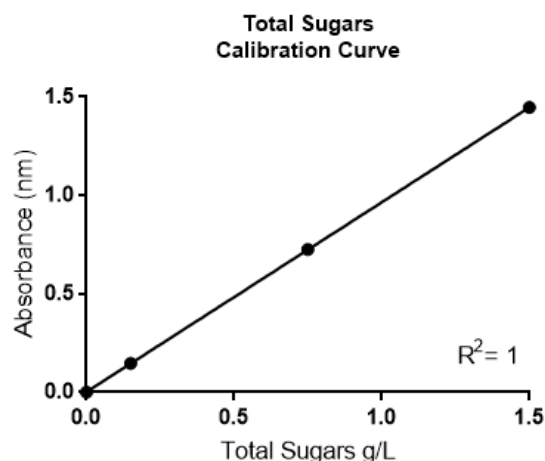
D-Glucose Automated Test Results

D-Glucose (g/L)	Replicates, n	Mean result (g/L)	Standard Deviation	%CV
0.075	4	0.075	0.0005	0.671
0.375	4	0.373	0.0022	0.581
0.750	4	0.754	0.0109	1.447



Total Sugars Automated Test Results

Total Sugars (g/L)	Replicates, n	Mean result (g/L)	Standard Deviation	%CV
0.150	4	0.149	0.0008	0.671
0.750	4	0.755	0.0050	0.581
1.500	4	1.536	0.0038	0.253



D-Fructose Automated Test Results

Total Sugars (g/L)	D-Glucose (g/L)	D-Fructose (g/L) (Total Sugars minus D-Glucose)
0.149	0.075	0.074
0.755	0.373	0.382
1.536	0.754	0.782

SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing
- Application support in relation to automated analyzers

Supporting documents can be found in the product page:

- Product Instructions
- D-Fructose / D-Glucose Liquid Ready™ Quick Reference Guide
- Mega-Calc™ D-Fructose / D-Glucose Liquid Ready™
- Safety data sheets (SDS)
- Certificate of analysis (CoA)



Contact us for more information: neogen.com/contact

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