D-FRUCTOSE / D-GLUCOSE (LIQUID READY™)

PRODUCT INSTRUCTIONS

SKU: 700007621
K-FGLQR

04/24

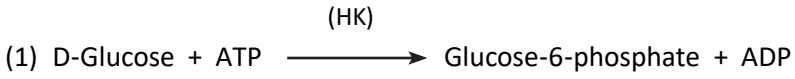
(50 Manual Assays per Kit) or
(500 Auto-Analyzer Assays per Kit)

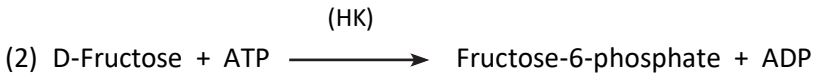
INTRODUCTION:

D-Fructose and D-Glucose are found in most plant products. They are present in significant quantities in honey, wine and beer, and a range of solid foodstuffs such as bread and pastries, chocolate and candies. In the wine industry, the sum of D-fructose and D-glucose, termed "total residual sugars", is a key parameter, as this represents the amount of sugar that is available to the yeast for the conversion into ethanol. Total residual sugar levels are monitored throughout fermentation and, after fermentation is complete, are adjusted to achieve the desired taste profile.

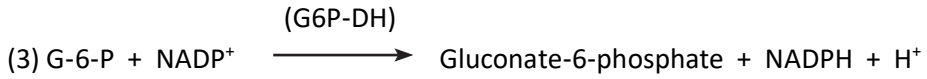
PRINCIPLE:

D-Glucose is phosphorylated by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) to glucose-6-phosphate (G-6-P) with the simultaneous formation of adenosine-5'-diphosphate (ADP) (1).





In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidised by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (3).



The amount of NADPH formed in this reaction is stoichiometric with the amount of D-glucose. It is the NADPH which is measured by the increase in absorbance at 340 nm. On completion of reaction (3), F-6-P is converted to G-6-P by phosphoglucose isomerase (PGI) (4).

The G-6-P formed reacts in turn with $NADP^+$ forming gluconate-6- phosphate and NADPH, leading to a further rise in absorbance that is stoichiometric with the amount of D-fructose.

SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific for D-Fructose and D-Glucose. They can be measured separately or as D-Fructose / D-Glucose (Total).
- The limit of detection (LOD) is 0.006 g/L, and the limit of quantification (LOQ) is 0.016 g/L (using a sample volume of 0.1 mL).
- The recommended working range is between 0.025 and 1.50 g/L (using a sample volume of 0.1 mL). This corresponds of 2.5 to 150 µg of D-Fructose / D-Glucose per assay.

INTERFERENCE:

No interfering compounds have been identified.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. After use, the reagents can be disposed of with the laboratory waste.

NOTE: For more information regarding the performance of this product please refer to the associated validation report available from the Megazyme website. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KIT CONTENTS:

Kits suitable for manual and automated format. The reagents are sufficient for performing 50 assays in manual format or 500 assays in auto-analyzer format. The kit contains:

Reagent 1 (2 x 50 mL): Buffer
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

Reagent 2 (2 x 12.5 mL): NADP, ATP, HK and G6P-DH
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

Reagent 3 (2 x 12.5 mL): PGI
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

Standard (5 mL): D-Glucose/D-Fructose standard
(1.5 g/L total sugars - 0.75 g/L of each sugar)
Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use.
Store at 4°C. See individual label for expiry date.

NOTE: The D-Glucose/D-Fructose standard solution is only assayed if there is some doubt about the accuracy of the spectrophotometer being used or if it is suspected that inhibition is being caused by substances in the sample. The concentration of D-Glucose/D-Fructose is determined directly from the extinction coefficient of NADPH.

PREPARATION OF REAGENT SOLUTIONS:

Bring all reagents to room temperature (20 - 25°C) before use.

MANUAL ASSAY PROCEDURE – D-FRUCTOSE + D-GLUCOSE (TOTAL):

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 37°C
Final volume: 3.10 mL
Sample solution: 0.025 g/L to 1.5 g/L (i.e. 2.5 - 150 µg of D-Glucose / D-Fructose per cuvette
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Sample
Reagent 1	2.0 mL	2.0 mL
Sample	-	0.1 mL
Distilled Water	0.1 mL	-
Mix*, incubate for ~ 3 mins at 20 - 37°C, then read the absorbances (A_1) Add Reagent 2 as described below		
Reagent 2	0.5 mL	0.5 mL
Reagent 3	0.5 mL	0.5 mL
Mix*, incubate for ~ 15 mins at 20-37°C, then read the absorbances (A_2).**		

* either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 min intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 min. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 3.

NOTE: The reagent blank value must be determined once for each run and subtracted from each sample result.

CALCULATION - D-FRUCTOSE + D-GLUCOSE (TOTAL):

NOTE These calculations can be simplified by using the *MegaCalc*™ tool, downloadable from the product page.

1. Calculation of the dilution factor (df)

Determine the dilution factor (df) of the optical densities due to the reagent volume.

$$df = \frac{\text{Sample volume [mL] + R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the **D-Glucose + D-Fructose** manual assay procedure:

$$df = \frac{0.1 + 2.0}{3.1} = 0.677$$

2. Calculation of the absorbance difference ΔA_{total}

The value of ΔA_{total} should as a rule be at least 0.1 absorbance units to achieve sufficiently accurate results.

$$\Delta A_{total} = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{blank}$$

It follows for the D-Glucose/ D-Fructose manual assay procedure:

$$\Delta A_{total} = (A_2 - 0.677 \times A_1)_{sample} - (A_2 - 0.677 \times A_1)_{blank}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the dilution factor. If volumes are changed, the performance may be affected.

3. Calculation of the D-Fructose + D-Glucose (total) content

The concentration of Total D-Glucose and D-Fructose can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{total} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of D-Glucose/D-Fructose [g/mol]
ε = extinction coefficient of NADPH at 340 nm [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for the total residual sugar manual assay procedure:

$$c = \frac{3.1 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{total} \quad [g/L]$$

$$= 0.8865 \times \Delta A_{total} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

4. Calculation of the residual sugar content in solid or semi-solid samples:

When analysing solid and semi-solid samples which are weighed for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

$$= \frac{c_{total}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ \ sample\ solution]} \times 100 \quad [g/100g]$$

MANUAL ASSAY PROCEDURE – SEQUENTIAL ASSAY - D-FRUCTOSE / D-GLUCOSE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 37°C
Final volume: 3.10 mL
Sample solution: 0.025 g/L to 1.5 g/L (i.e. 2.5 - 150 µg of D-Fructose / D-Glucose per cuvette
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Sample
Reagent 1	2.0 mL	2.0 mL
Sample	-	0.1 mL
Distilled Water	0.1 mL	-
Mix*, incubate for ~ 3 mins at 20 - 37°C, then read the absorbances (A_1) Add Reagent 2 as described below:		
Reagent 2	0.5 mL	0.5 mL
Mix*, incubate for ~5 mins at 20 - 37°C, then read the absorbances (A_2) Add Reagent 3 as described below:		
Reagent 3	0.5 mL	0.5 mL
Mix*, incubate for ~15 mins at 20 - 37°C, then read the absorbances (A_3). **		

* either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 min intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 min. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 3.

NOTE: The reagent blank value must be determined once for each run and subtracted from each sample result.

CALCULATION – SEQUENTIAL ASSAY - D-FRUCTOSE / D-GLUCOSE:

NOTE: These calculations can be simplified by using the *MegaCalc*™ tool, downloadable from the product page.

DETERMINATION OF D-GLUCOSE CONTENT:

1. Calculation of the dilution factor

Determine the dilution factor (df) of the optical densities due to the reagent volume.

$$df = \frac{\text{Sample volume [mL] + R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the D-Glucose manual assay procedure:

$$df = \frac{0.1 + 2.0}{2.6} = 0.808$$

2. Calculation of the absorbance difference $\Delta A_{D\text{-glucose}}$

The value of $\Delta A_{D\text{-glucose}}$ should as a rule be at least 0.1 absorbance units to achieve sufficiently accurate results.

$$\Delta A_{D\text{-glucose}} = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{blank}$$

It follows for the D-Glucose manual assay procedure:

$$\Delta A_{D\text{-glucose}} = (A_2 - 0.808 \times A_1)_{sample} - (A_2 - 0.808 \times A_1)_{blank}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the reagent factor. If volumes are changed, the performance may be affected.

3. Calculation of the D-Glucose content

The concentration of D-Glucose can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{D\text{-glucose}} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of D-Glucose [g/mol]
ε = extinction coefficient of NADPH at 340 nm [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for the D-Glucose manual assay procedure:

$$c = \frac{2.6 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{D\text{-glucose}} \quad [g/L]$$

$$= 0.7435 \times \Delta A_{D\text{-glucose}} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

4. Calculation of the D-Glucose content in solid or semi-solid samples:

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

$$= \frac{c_{D\text{-glucose}}\ [g/L\ \text{sample solution}]}{\text{weight}_{sample}\ [g/L\ \ \text{sample solution}]} \times 100 \quad [g/100g]$$

DETERMINATION OF D-FRUCTOSE CONTENT:

1. Calculation of the dilution factor

Determine the dilution factor (df) of the optical densities due to the reagent volume.

$$df = \frac{\text{Sample volume [mL]} + \text{R1 volume [mL]} + \text{R2 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the D-Fructose manual assay procedure:

$$df = \frac{0.1 + 2.0 + 0.5}{3.1} = 0.839$$

2. Calculation of the absorbance difference $\Delta A_{D\text{-fructose}}$

The value of $\Delta A_{D\text{-fructose}}$ should as a rule be at least 0.1 absorbance units to achieve sufficiently accurate results.

$$\Delta A_{D\text{-fructose}} = (A_3 - df \times A_2)_{sample} - (A_3 - df \times A_2)_{blank}$$

It follows for the D-Fructose manual assay procedure:

$$\Delta A_{D\text{-fructose}} = (A_3 - 0.839 \times A_2)_{sample} - (A_3 - 0.839 \times A_2)_{blank}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the reagent factor. If volumes are changed, the performance may be affected.

3. Calculation of the D-Fructose content

The concentration of D-Fructose can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{D\text{-fructose}} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of D-Fructose [g/mol]
ε = extinction coefficient of NADPH at 340 nm [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for the D-Fructose standard manual assay procedure:

$$c = \frac{3.1 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{D\text{-fructose}} \quad [g/L]$$

$$= 0.8865 \times \Delta A_{D\text{-fructose}} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

4. Calculation of the D-Fructose content in solid or semi-solid samples:

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of D-Fructose

$$= \frac{c_{D\text{-fructose}}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ \ sample\ solution]} \times 100 \quad [g/100g]$$

AUTO-ANALYZER ASSAY PROCEDURE:

This kit has been designed for biochemistry analyzers and can be adapted to most instruments. A sample method is shown below (validated on the Awareness ChemWell®-T analyzer).

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).

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	
Parameter	**Details**
Wavelength	340/405 nm (primary/secondary)
Temperature	20 - 37°C
Test	End-point test with following test sequence: – Add Reagent 1 **[0.2 mL]** – Add Sample or Calibrator **[0.01 mL]** – Pre-incubate for 1-3 min [20-37°C] – Measure A_1 at 340/405 nm – Add Reagent 2 **[0.05 mL]** – Incubate 5 mins at [20-37°C] – Measure A_2 at 340/405 nm – Calculate $A_2 – A_1$ against calibration curve
Calibration	Calibrate using 2 – 4 calibrators ranging from 0 – 0.75 g/L. The calibration curve is linear. An example of how to use the standard supplied with the kit to create a calibration curve is shown below: Calibrator 1 0 g/L (use distilled water) Calibrator 2 0.075 g/L (dilute Standard 10-fold) Calibrator 3 0.375 g/L (dilute Standard 2-fold) Calibrator 4 0.75 g/L (use Standard as-is) *Perform all dilutions with distilled water.*

D-Fructose / D-Glucose (Total)	
Parameter	**Details**
Wavelength	340/405 nm (primary/secondary)
Temperature	20 - 37°C
Test	End-point test with following test sequence: – Add Reagent 1 **[0.2 mL]** – Add Sample or Calibrator **[0.01 mL]** – Pre-incubate 1-3 min [20 - 37°C] – Measure A_1 at 340/405 nm – Add Reagent 2 **[0.05 mL]** – Add Reagent 3 **[0.05 mL]** – Incubate 15 mins at [20 - 37°C] – Measure A_2 at 340/405 nm – Calculate $A_2 - A_1$ against calibration curve
Calibration	Calibrate using 2 – 4 calibrators ranging from 0 – 1.5 g/L. The calibration curve is linear. An example of how to use the standard supplied with the kit to create a calibration curve is shown below: Calibrator 1 0 g/L (use distilled water) Calibrator 2 0.15 g/L (dilute Standard 10-fold) Calibrator 3 0.75 g/L (dilute Standard 2-fold) Calibrator 4 1.5 g/L (use Standard as-is) *Perform all dilutions with distilled water.*

SAMPLE PREPARATION:

1. Sample dilution

The amount of D-Fructose / D-Glucose present in the sample should range from 0.025 g/L to 1.5 g/L. If the value of ΔA is too low (e.g. <0.1), weigh more sample or decrease the dilution. If the value ΔA is too high (e.g. >2.5 in total), increase the dilution in distilled water.

Dilution Table

Estimated Concentration of D-Fructose + D-Glucose (g/L)	Dilution with Water	Dilution factor (F)
<1.5	No Dilution required	1
1.5 – 15	1mL sample + 9 mL water	10
15 -150	1mL sample + 99 mL water	100

2. General sample preparation guide

- Clear, slightly coloured and approximately neutral, liquid samples at a concentration up to 1.5 g/L can be used directly in the assay.
- Turbid samples should be filtered or centrifuged.
- Acidic samples (pH <3.0) should be neutralised to approximately pH 7.0.
- Samples containing carbon dioxide should be degassed by gentle agitation or stirring with a glass rod.
- Solid samples should be homogenised, extracted in water and filtered or centrifuged if necessary.
- Strongly coloured samples should be treated by the addition of 0.2 g of polyvinylpolypyrrolidone (PVPP) per 10 mL of sample in a tube. Shake the tube vigorously for 5 minutes and then filter through filter paper.
- Deproteinize samples using the Megazyme Carrez Clarification Kit (K-CARREZ).
- Remove fat using the Megazyme Carrez Clarification Kit (K-CARREZ).

3. Suggested sample preparation examples

(a) Determination of D-Fructose / D-Glucose in wine. Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g. *Typically, a 3-fold dilution in distilled water is required.*

(b) Determination of D-Fructose / D-Glucose in fruit juice (e.g. apple juice/orange juice). Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of juice for 5 minutes at 15,000 g. *Typically, a 50-fold dilution in distilled water is required.*

(c) Determination of D-Fructose / D-Glucose in breakfast cereals. Homogenize samples in a blender. Accurately weigh 1 g of the sample into a 50 mL volumetric flask and make up to the mark using

distilled water. Add a stir bar and stir for 5 minutes to allow extraction of monosaccharides. Finally, filter using filter paper and use the clear filtrate for the assay. *Typically, no further dilution is required.*

(d) Determination of D-Fructose / D-Glucose in confectionary products (e.g. sour jellies). Accurately weigh 4 g of confectionary into a 100 mL graduated cylinder and adjust to 100 mL with distilled water. Quantitatively transfer the contents of the cylinder to a blender and blend for 30 seconds, or until homogeneous. Allow any foam formed to settle and the liquid can then be used in the assay protocol with no further treatment. *Typically, a 10-fold dilution in distilled water is required.*

(e) Determination of D-Fructose / D-Glucose in dairy products (e.g. liquid fruit yogurt). Pipette 10 mL of sample into a 50 mL volumetric flask, add the following solutions and mix after each addition: 5 mL of Carrez I solution, 5 mL of Carrez II solution and 10 mL of NaOH solution (100 mM). Fill up to the mark with distilled water, mix and filter using a paper filter. Alternatively, follow the protocol in the Megazyme Carrez Clarification Kit (K-CARREZ). *Typically, no further dilution is required.*

(f) Determination of D-Fructose / D-Glucose in fruit and vegetables (e.g. grapes). Accurately weigh 30 g of grapes into a 100 mL graduated cylinder and adjust to 100 mL using distilled water. Quantitatively transfer the contents of the cylinder to a blender and blend for 30 seconds, or until homogeneous. Filter solution using a paper filter and use a clear filtrate for the assay. *Typically, a 100-fold dilution in distilled water is required.*

IMPORTANT NOTE: Users should perform in-house matrix validation prior to routine use. This process will highlight any problematic matrices encountered. The above are suggested sample preparation examples only. If you have questions about these or other matrices, please contact your local sales representative for support.

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:

Quick Reference Guide
MegaCalc™
Safety Data Sheets (SDS)
Certificates Of Analysis (COA)
Validation Report

Contact us for more information: neogen.com/contact

Without guarantee

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents. It is the user's responsibility to perform in-house matrix validation work prior to routine use.

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