

FT-U67120

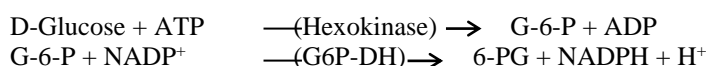
## D-Glucose Assay

### Product Description

**Catalog number:** U67120, 1 kit, 32 tests

**Name:** **D-Glucose Assay** (UV HK method)

#### Principle



Ref: Schmidt, F.H (1961) Die enzymatische Bestimmung von Glucose und Fructose nebeneinander, Klinische Wochenschrift 39, 1244-1247

#### Assay characteristics

Wavelength: 340 nm (NADH)  $\epsilon = 6.31 \times \text{mmol}^{-1} \times \text{cm}^{-1}$   
 Light path (cuvettes) : 1.00 cm (glass ; plastic)  
 Temperature: +20 to +25°C  
 Assay volume 3.020 ml  
 Measurement: against air or against water  
 Sample solution: 1 to 100 µg D-glucose in 0.1 to 2.0 ml sample solution

#### Reagents

**#1** : #U6712a - Powder mixture with triethanolamine buffer, pH 7.6, approx. 80 mg NADP, approx 190 mg ATP, magnesium sulfate (for stability see pack label).

*Dissolve contents of bottle 1 with 31 ml redist. Water.* The solution is stable for 1 month at +2 to +8°C, or 2 months at -15 to -25°C.

**#2** : #U6712b - 0.7 ml hexokinase (HK) / glucose-6-phosphat dehydrogenase (G6P-DH) suspension (approx. 200 U / 100 U) in ammonium sulfate (for stability see pack label).

*The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.

In addition (not contained in the kit) :

Standard solution D-glucose, anhydrous, ultrapure, 0.5 g/l for test control only.

**Safety:** The reagents for the determination of D-glucose are not hazardous.

The general safety rules for the work in chemical laboratories should be applied.

After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

#### Procedure

Pipette into cuvettes	Blank	Standard <sup>1</sup>	Sample <sup>2</sup>	Rerun assay <sup>3</sup>	Assay with internal standard <sup>4</sup>	High sensitive assay <sup>5</sup>
<b>Reagent #1</b> Tea Buffer NADP, ATP	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
<b>Sample solution<sup>6</sup></b> (e.g 0.05 to 0.5 g D-glucose/l)	-	-	<b>0.100 ml</b>	<b>0.200 ml</b>	<b>0.100 ml</b>	<b>2.000 ml</b>
Sample solution <sup>6</sup> (e.g 0.5 g D-glucose/l)	-	0.100 ml	-	-	0.100 ml	-
Redist. water	2.000 ml	1.900 ml	1.900 ml	1.800 ml	1.800 ml	-
<b>Mix<sup>7</sup>, after approx. 3 min read the absorbance (A<sub>1</sub>). Add:</b>						
<b>Reagent #2</b> HK/G6P-DH suspension	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
<b>Mix<sup>7</sup>, after approx. 10-15 min read the absorbance (A<sub>2</sub>). Repeat absorbance reading after another 2 min<sup>8</sup></b>						

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## Notes :

- <sup>1</sup> Run a « standard » to see « accidents » in analysis. The measurement of the standard is not necessary for calculating results.
- <sup>2</sup> This assay together with the blank is a single determination.
- <sup>3</sup> In the case of a double determination, run two assays with different sample volumes. The absorbance differences measured have to be proportional to the sample volumes. Calculate with the resp. v.
- <sup>4</sup> Recovery =  $[(\Delta_{\text{sample+standard}} - \Delta_{\text{standard}}) \times 100 \text{ \%}]$
- <sup>5</sup> Assay recommended in the case of trace level compound analysis, with sample volume increased up to 2.000 ml (0.0004 to 0.05 g D-glucose/l)
- <sup>6</sup> Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
- <sup>7</sup> e.g with a plastic spatula, or after closing the cuvette with Parafilm™ #601181 (trademark of American Can Co., Greenwich Ct.,USA).
- <sup>8</sup> The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read absorbances until the absorbances increase constantly over e.g 2 min. Extrapolate absorbances to the time of the addition of HK/GP-DH (suspension #2)

## Calculation :

$$\Delta A = (A_2 - A_1)_{\text{sample, resp. standard}} - (A_2 - A_1)_{\text{blank}}$$

$$c = (V \times MW \times \Delta A) / (\epsilon \times d \times v \times 1000) \text{ [g D-glucose/l sample solution]}$$

$$c = (3.020 \times 180.16 \times \Delta A) / (6.3 \times 1.00 \times 0.100 \times 1000) = \mathbf{0.8636 \times \Delta A \text{ [g D-glucose/l sample solution]}}$$

If the sample has been diluted during preparation, multiply the result with dilution factor F.

When analyzing samples which are weighted out for sample preparation, calculate the content from the amount weighed :

$$\text{Content}_{\text{D-glucose}} = \frac{C_{\text{D-glucose}} \text{ [g/l sample solution]}}{\text{weight}_{\text{sample}} \text{ [in g/l sample solution]}} \times 100 \text{ [g/100 g]}$$

## Sample preparation :

1. Dilute *clear, colorless and neutral liquid samples* to get a sample solution with 0.05 to 0.5 g D-glucose/l.
2. Filter or centrifuge *turbid solutions*, dilute (see pt. 1).
3. Degas *samples containing carbon dioxide*, e.g by filtration, or add NaHCO<sub>3</sub> till the solution is slightly alkaline, dilute (see pt. 1).
4. Adjust *acid (esp. Slightly colored) solutions* with KOH or NaOH to approx. pH 7, incubate a few minutes, or dilute (see pt. 1) without pH adjustment in the case of colorless samples.
5. Treat « *strongly colored solutions* » used undiluted with PVPP or polyamide, e.g 1 g/100 ml, mix, incubate a few minutes, filter.
6. Crush (corn size < 0.3 mm) or homogenize *solid or semi-solid (pasty) samples*, extract with water, or dissolve in water, filter and dilute (see pt. 1) if necessary.
7. Extract *fat containing samples* with hot water at temperature above the melting point, e.g. in a 100 ml volumetric flask. Adjust to +20°C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter. Alternatively, clarify with Carrez reagents (wich can be recommended).
8. Clarify *samples containing protein* with Carrez reagents :  
Weigh sufficient quantity of solid or pasty sample into 100 ml volumetric flask, add approx. 60 ml water. Or pipette liquid sample into 100 ml volumetric flask containing approx. 60 ml water. Add, and mix after each addition, 5 ml Carrez-I-solution (3.60 g K<sub>4</sub>[Fe(CN)<sub>6</sub>] x 3H<sub>2</sub>O = potassium hexacyanoferrate(II)/100 ml), 5 ml Carrez-II-solution (7.20 g ZnSO<sub>4</sub> x 7H<sub>2</sub>O = zinc sulfate hepta-hydrate/100 ml). Adjust to pH 7.5 to 8.5 by the addition of e.g 10 ml NaIH (0.1M). Fill the flask to the mark, mix and filter.
9. *Deproteinize samples with perchloric acid only in the absence of sucrose and maltose.*

## Assay performance

- Specificity :** Specific for D-glucose. In the analysis of commercial D-glucose and D-glucose monohydrate results of < 100 % have to be expected because the materials absorb moisture.
- Sensitivity :** 0.2 mg/l ( $\Delta A = 0.005$  ;  $v=2.000$  ml ;  $V = 3.020$  ml)
- Detection limit :** 0.4 mg/l ( $\Delta A = 0.010$  ;  $v=2.000$  ml ;  $V = 3.020$  ml)
- Linearity :** 1  $\mu$ g / assay ( $v=2.000$  ml ;  $V = 3.020$  ml)  
to 100  $\mu$ g/assay ( $v=0.100$  ml ;  $V = 3.020$  ml)
- Precision :** +/- 0.005 absorbance units  
CV = approx. 1 to 2 % ;  
Fruit juice :  $r = 0.42 + 0.027 \times C_{D\text{-glucose in g/l}}$  [g/l]  
 $R = 1.0 + 0.042 \times C_{D\text{-glucose in g/l}}$  [g/l]  
Diet beer :  $r = 0.30$  g/l,  $R = 1.22$  g/l ( $x = 10$  g/l)  
Wine :  $r = 0.056 \times C_{D\text{-glucose in g/l}}$  [g/l]
- Interferences :** None known
- Technical Information :** The reagents can also be used for the determination of D-fructose (with additional PGI) and sucrose (with additional  $\beta$ -fructosidase).
- Reference :**  $\square$   
The method is contained in the Austrian, German, Italian, Swiss food laws and in European regulation.  
Recommended e.g by AOC, IFU, AIJN, MEBAK, OICCC, OIV. Standardized by DIN, EN, GOST, NEN, NF.

## Additional Information

### General

D-Glucose is present in significant quantities in foods (fruit juices, honey, milk and dairy products, wine, cider beer and other beverages, and in foodstuffs). It is abundantly found in the chemistry composition of various plants, vegetables and fruits. It occurs as a free sugar or in a range of di-, oligo- and poly-saccharides, such as starch, cellulose, etc.

This assay #U6712 uses the enzyme hexokinase (HK) to catalyze the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate and adenosine diphosphate (ADP). In the presence of NAD, the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), oxidizes glucose-6-phosphate to 6-phosphogluconate. The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

This assay allows to dose D-Glucose in a variety of foodstuffs and other materials (refer below to 'sample preparation' for specific samples). The reagents can also be used for the determination of D-fructose (with additional PGI) and sucrose (with additional  $\beta$ -fructosidase).

### Sample preparation : $\square$

If the sample has one of the characteristics below, which hamper the test, please follow the corresponding sample preparation procedure:

**Note:** for *serum and other biological fluids samples*, we recommend the Assay kit #BD1850

- clear, colorless and neutral liquid samples** can be used directly in the assay (dilute to get a sample solution with 0.05 to 0.5 g D-glucose/l).
- Filter or centrifuge **turbid solutions**, dilute if necessary (see pt. 1).
- Degas **samples containing carbon dioxide**, e.g by filtration, or add  $\text{NaHCO}_3$  till the solution is slightly alkaline, dilute (see pt. 1).
- Adjust **acid (esp. slightly colored) solutions** with KOH or NaOH to approx. pH 7.5, incubate a few minutes. (for example red wine, cider or coloured fruit juice)
- Treat « **strongly colored solutions** » used undiluted with PVPP or polyamide, e.g 1 g/100 ml, mix, incubate a few minutes, filter. An sample blank, i.e. sample with no HK/G2PDH, should be performed to adjust the photometer.

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6. Crush (corn size < 0.3 mm) or homogenize **solid or semi-solid (pasty) samples**, extract with water, or dissolve in water, filter and dilute (see pt. 1) if necessary.

7. Extract **fat containing samples** with hot water at temperature above the melting point, e.g. in a 100 ml volumetric flask. Adjust to +20°C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter. Alternatively, clarify with Carrez reagents (which can be recommended).

8. Clarify **samples containing protein** with Carrez reagents (normal use\*):

Weigh sufficient quantity of solid or pasty sample into 100 ml volumetric flask, add approx. 60 ml water. Or pipette liquid sample into 100 ml volumetric flask containing approx. 60 ml water. Add (mix after each addition): 5 ml Carrez-I-solution (3.60 g  $K_4[Fe(CN)_6] \times 3H_2O$  = potassium hexacyanoferrate(II)/100 ml), 5 ml Carrez-II-solution (7.20 g  $ZnSO_4 \times 7H_2O$  = zinc sulfate hepta-hydrate/100 ml). Adjust to pH 7.5 to 8.5 by the addition of e.g 10 ml NaOH (0.1M). Fill the flask to the 100ml mark, mix and filter.

\*Alternatively, deproteinize samples with perchloric acid or with trichloroacetic acid only in the absence of sucrose and maltose (these disaccharides are fully or partially hydrolyzed with the release of D-Glucose).

[Specific applications/procedures](#), i.e. foodstuffs, preserves and vegetables or fruits, Milk, Fermentation samples, total D-Glucose after acid hydrolysis.

## Other information :

For in vitro R&D use only

Other biochemistry kits:

-Agroalimentary: Acetic acid, Ascorbic a., Citric a., D-Gluconic a., D-Glutamic a., L-Lactic a., D-/L-Lactic a., D-Malic a., L-Malic a., Starch, D-Glucose, D-Glucose / D-Fructose, D-Glucose / Sucrose, D-Glucose / D-Fructose / Sucrose, Glycerol, Lactose /Galactose

-Clinical chemistry kits: Glucose assay for serum and plasma #BD1850

Please contact InterBioTech – Interchim for any other information

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