UV method for approx. 32 assays

For *in vitro* use only
Store between +2 and +8 °C

The method is contained in the Austrian, German, Italien, Swiss food laws and in European regulation. Recommended e.g. by AOAC, IFU, AIJN, MEBAK, OICCC, OIV. Standardized by DIN, EN, GOST, NEN, NF.

Principle

D-Glucose + ATP ——— Hexokinase ⟶ G-6-P + ADP
G-6-P + $NADP^+$ ——— G6P-DH ⟶ 6-PG + NADPH + H^+

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

Assay performance

Wavelength: 340 nm (NADPH), ε = 6.3 l x $mmol^{-1}$ x 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 0.100 to 2.000 ml sample solution.

Reagents

1: Powder mixture with triethanolamine buffer, pH approx. 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, resp. 2 months at -15 to -25 °C.

2: Approx. 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.

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[1]**	**Sample[2]**	**Rerun assay[3]**	**Assay with internal standard[4]**	**High sensitive assay[5]**
Tea buffer, NADP, ATP, solution # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
Sample solution[6] (e.g. 0.05 to 0.5 g D-glucose/l)	**-**	**-**	**0.100 ml**	**0.200 ml**	**0.100 ml**	**2.000 ml**
Standard solution[6] (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	-
Mix[7], after approx. 3 min read the absorbances (A_1). Add:						
HK/G6P-DH suspension # 2	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix[7], after approx. 10 to 15 min read the absorbances (A_2). Repeat absorbance reading after another 2 min[8]						

Notes

1 Run a „standard“ to see „accidents“ in analysis. The measurement of the standard is not necessary for calculating results.
2 This assay together with the blank is a single determination.
3 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.
4 Recovery = $[(\Delta A_{Sample + standard} - \Delta A_{sample}) / \Delta A_{standard}] \times 100$ [%].
5 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).
6 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
7 e.g. with a plastic spatula, or after closing the cuvette with Parafilm® (American Can Co., Greenwich Ct., USA).
8 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/G6P-DH (suspension # 2).

Calculation

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

c = (V x MW x ΔA) / (ε x d x v x 1000) [g D-glucose/l sample solution]
c = (3.020 x 180.16 x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **0.8636 x ΔA [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 weighed out for the sample preparation, calculate the content from the amount weighted:

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

Sample preparation:

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

1. Dilute *clear, colorless and almost 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 $NaHCO_3$ 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 a temperature above the melting point of fat, 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*:* 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 $K_4[Fe(CN)_6] \times 3H_2O$ = potassium hexa-cyanoferrate(II)/100 ml), 5 ml Carrez-II-solution (7.20 g $ZnSO_4 \times 7\ H_2O$ = zinc-sulfate hepta-hydrate/100 ml). Adjust to pH 7.5 to 8.5 by the addition of e.g. 10 ml NaOH (0.1 M). Fill the flask to the mark, mix and filter.
9. *Deproteinize samples* with perchloric acid only in the absence of sucrose and maltose.

Assay characteristics

1. *Specific*ity: 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.
2. *Sensitivity:* 0.2 mg (ΔA = 0.005; v = 2.000 ml; V = 3.020 ml)
3. *Detection limit*: 0.4 mg (ΔA = 0.010; v = 2.000 ml; V = 3.020 ml)
4. *Linearity* 1 µg/assay (v = 2.000 ml; V = 3.020 ml)
   to 100 µg/assay (v = 0.100 ml; V = 3.020 ml)
5. *Precision:* ΔA = +/- 0.005 absorbance units
   CV = approx. 1 to 2 %
   Fruit juice: $r = 0.42 + 0.027 \times c_{\text{D-glucose in g/l}}$ [g/l]
   $R = 1.0 + 0.042 \times c_{\text{D-glucose in g/l}}$ [g/l]
   Dietbeer: x = 10 g/l r = 0,30 g/l
   R = 1,22 g/l
   Wine: $r = 0.056 \times c_{\text{D-glucose in g/l}}$ [g/l]
6. *Interferences:* None known
7. *Technical information:* the reagents can also be used for the determination of D-fructose (with additional PGI) and sucrose (with additional β-fructosidase).