D-Glucose/D-Fructose

UV-method

for the determination of D-Glucose and D-fructose in foodstuffs and other materials

Fast method for analysis of wine: see under pt. 10

BOEHRINGER MANNHEIM / R-BIOPHARM
Enzymatic BioAnalysis / Food Analysis

For *in vitro* use only

Store at 2-8°C

Cat. No. 10 139 106 035

Test-Combination for 27 determinations each

For recommendations for methods and standardized procedures see references (A 2, B 2, C 2, D 2)

Principle (Ref. A 1)

D-Glucose and D-fructose are phosphorylated to D-glucose-6-phosphate (G-6-P) and D-fructose-6-phosphate (F-6-P) by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP) (1,2).

(1) D-Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

(2) D-Fructose + ATP $\xrightarrow{HK}$ F-6-P + ADP

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

(3) G-6-P + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-phosphate + NADPH + H^+

The amount of NADPH formed in this reaction is stoichiometric to the amount of D-glucose. NADPH is measured by the increase of its light absorbance at 334, 340 or 365 nm.

On completion of reaction (3), F-6-P is converted to G-6-P by phosphoglucose isomerase (PGI) (4).

(4) F-6-P $\xrightleftharpoons{PGI}$ G-6-P

G-6-P reacts in turn with NADP forming D-gluconate-6-phosphate and NADPH. The amount of NADPH obtained in this reaction is stoichiometric to the amount of D-fructose. The increase in NADPH is measured by means of its light absorbance.

The Test-Combination contains

1. Bottle 1 with approx. 5 g of powder mixture, consisting of: triethanolamine buffer, pH approx. 7.6; NADP, approx. 64 mg; ATP, approx. 160 mg; magnesium sulfate
2. Bottle 2 with approx. 0.7 ml suspension, consisting of: hexokinase, approx. 200 U; glucose-6 phosphate dehydrogenase, approx. 100 U
3. Bottle 3 with approx. 0.7 ml suspension phosphoglucose isomerase, approx. 490 U
4. Bottle 4 with D-glucose assay control solution for assay control purposes (measurement of the assay control solution is not necessary for calculating the results.) The assay control solution does not contain D-fructose because of its insufficient stability in aqueous solutions. Use the assay control solution undiluted. (Expiry date: see pack label)

Preparation of solutions

1. Dissolve contents of bottle 1 in 27 ml of redist. water.
2. Use contents of bottle 2 undiluted.
3. Use contents of bottle 3 undiluted.

Stability of reagents

The contents of bottle 1 are stable at 2-8°C (see pack label).
Solution 1 is stable for 4 weeks at 2-8°C, and for 2 months at −20 to −25°C.
Bring solution 1 to 20-25°C before use.
The contents of bottles 2 and 3 are stable at 2-8°C (see pack label).

Procedure

Wavelength:[1] 340 nm, Hg 365 nm or Hg 334 nm
Glass cuvette:[2] 1.00 cm light path
Temperature: 20-25°C
Final volume: D-glucose 3.020 ml
D-fructose 3.040 ml
Read against air (without a cuvette in the light path) or against water
Sample solution: 1-100 µg of D-glucose and D-fructose per assay[3]
(in 0.100-2.000 ml sample volume)

Pipette into cuvettes	Blank	Sample
solution 1	1.000 ml	1.000 ml
sample solution*	-	0.100 ml
redist. water	2.000 ml	1.900 ml
Mix**, and read absorbances of the solutions (A_1) after approximately 3 min and start reaction by addition of:		
suspension 2	0.020 ml	0.020 ml
Mix**, wait for the end of the reaction (approx. 10-15 min), and read the absorbances of the solutions (A_2). If the reaction has not stopped after 15 min, continue to read the absorbances at 2 min intervals until the absorbances increase constantly over 2 min***. Add		
suspension 3	0.020 ml	0.020 ml
Mix**, read absorbances of the solutions after 10-15 min (A_3).		

* Rinse the enzyme pipette or the pipette tip of the piston pipette with sample solution before dispensing the sample solution.

** For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm (trademark of the American Can Company, Greenwich, Ct., USA)

*** "Creep reactions" occur very occasionally. They are mostly brought about by the contents of the sample solution, such as enzymes or coloring agents. These interfering substances may be removed during sample preparation.

If the absorbance A_2 increases constantly, extrapolate the absorbances to the time of the addition of suspension 2 (HK/G6P-DH).

Determine the absorbance differences (A_2-A_1) for both, blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-glucose}}$.

Determine the absorbance differences (A_3-A_2) for both, blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-fructose}}$.

The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently precise results (see "Instructions for performance of assay" and "Sensitivity and detection limit", pt. 4).

Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

V = final volume [ml]

v = sample volume [ml]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path [cm]

ε = extinction coefficient of NADPH at:
340 nm = 6.3 [l × $mmol^{-1}$ × cm^{-1}]
Hg 365 nm = 3.5 [l × $mmol^{-1}$ × cm^{-1}]
Hg 334 nm = 6.18 [l × $mmol^{-1}$ × cm^{-1}]

It follows for D-glucose:

$$c = \frac{3.020 \times 180.16}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A_{D\text{-glucose}} = \frac{5.441}{\varepsilon} \times \Delta A_{D\text{-glucose}}$$

[g D-glucose/l sample solution]

for D-fructose:

$$c = \frac{3.040 \times 180.16}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A_{D\text{-fructose}} = \frac{5.477}{\varepsilon} \times \Delta A_{D\text{-fructose}}$$

[g D-fructose/l sample solution]

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

1 The absorption maximum of NADPH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm **or** 334 nm.

2 If desired, disposable cuvettes may be used instead of glass cuvettes.

3 See instructions for performance of assay

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When analyzing solid and semi-solid samples which are weighed out for sample preparation, the result is to be calculated from the amount weighed.

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

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

1. Instructions for performance of assay

The amount of D-glucose + D-fructose present in the assay has to be between 2 µg and 100 µg (measurement at 365 nm) or 1 µg and 50 µg (measurement at 340, 334 nm), respectively. In order to get a sufficient absorbance difference, the sample solution is diluted to yield a D-glucose + D-fructose concentration between 0.15 and 1.0 g/l or 0.08 and 0.5 g/l respectively.

Dilution table

Estimated amount of D-glucose + D-fructose per liter measurement at		Dilution with water	Dilution factor F
340 or 334 nm	365 nm		
< 0.5 g	< 1.0 g	-	1
0.5-5.0 g	1.0-10.0 g	1 + 9	10
5.0-50 g	10.0-100 g	1 + 99	100
> 50 g	> 100 g	1 + 999	1000

If the measured absorbance difference (ΔA) is too low (e.g. < 0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) *or* the sample volume to be pipetted into the cuvette can be increased up to 2.000 ml. The volume of water added must then be reduced so as to obtain the same final volume in the assays for sample and blank. The new sample volume v must be taken into account in the calculation.

2. Technical information

If the ratio D-glucose to D-fructose in the sample is higher than e.g. 10:1, the precision of the D-fructose determination is impaired. In this case, as much as possible of the D-glucose should be removed by means of glucose oxidase in the presence of oxygen from the air. (For details see pt 11.)

If the concentration of D-fructose in the sample solution is higher or much higher than the concentration of D-glucose, both, D-fructose and D-glucose can be determined with high precision when the determinations are done in separate assays with different sample solutions. For details see the dilution table.

3. Specificity (Ref. A 1)

The method is specific for D-glucose and D-fructose.

In the analysis of commercial water-free D-glucose (molecular weight 180.16), D-glucose monohydrate (molecular weight 198,17) and D-fructose, results of < 100% have to be expected because the materials absorb moisture. (Commercial D-fructose may also contain D-glucose.)

4. Sensitivity and detection limit (Ref. A 1.4, A 1.5)

The smallest differentiating absorbance for the procedure is 0.005 absorbance units. This corresponds to a maximum sample volume v = 2.000 ml and measurement at 340 of a D-glucose or D-fructose concentration of 0.2 mg/l sample solution (if v = 0.100 ml, this corresponds to 4 mg/l sample solution).

The detection limit of 0.4 mg D-glucose, resp. D-fructose/l is derived from the absorbance difference of 0.010 (as measured at 340 nm) and a maximum sample volume v = 2.000 ml.

5. Linearity

Linearity of the determination exists from approx. 1 µg D-glucose + D-fructose/assay (0.4 mg D-glucose + D-fructose/l sample solution; sample volume v = 2.000 ml) to 100 µg D-glucose + D-fructose/ assay (1 g D-glucose + D-fructose/l sample solution; sample volume v = 0.100 ml).

6. Precision

In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of v = 0.100 ml and measurement at 340 nm, this corresponds to a D-glucose or D-fructose concentration of approx. 4-8 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.04-0.08 g/100 g can be expected.)

The following data have been published in the literature:

D-Glucose	CV = 1.2 %	blood	(Ref. A 1.2)
	CV = 1.8 %	blood	(Ref. A 1.4)
D-Fructose:	CV = 1.5 %	chocolate	(Ref. A 1.3)
	CV = 1.5 %	drinks, juices, honey	(Ref. A 1.5)

Fruit juice:
D-glucose: r = 0.42 + 0.027 × ($c_{\text{D-glucose}}$ in g/l) g/l
R = 1.0 + 0.042 × ($c_{\text{D-glucose}}$ in g/l) g/l
D-fructose: r = 0.15 + 0.033 × ($c_{\text{D-fructose}}$ in g/l) g/l
R = 1.05 + 0.045 × ($c_{\text{D-fructose}}$ in g/l) g/l
For further data see references (Ref. A 2.9)

Wine: $r = 0.056 \times x_i$ $R = 0.12 + 0.076\ x_i$
x_i = D-glucose resp. D-fructose content in g/l (Ref. A 2.17, 2.18)

D-Glucose in diet beer:
x = 1.0 g/100 ml r = 0.030 g/100 ml $s_{(r)} = \pm 0.011$ g/100 ml
R = 0.122 g/100 ml $s_{(R)} = \pm 0.043$ g/100 ml
For further data see references (Ref. B 2.2)

Liquid whole egg:
D-glucose: x = 0.44 g/100 g r = 0.073 g/100 g $s_{(r)} = \pm 0.026$ g/100 g
R = 0.106 g/100 g $s_{(R)} = \pm 0.037$ g/100 g
D-fructose: x = 6.72 g/100 g r = 0.587 g/100 g $s_{(r)} = \pm 0.207$ g/100 g
R = 0.748 g/100 g $s_{(R)} = \pm 0.264$ g/100 g
For further data see references (Ref. C 2.4)

7. Recognizing interference during the assay procedure

7.1 If the conversion of D-glucose and of D-fructose has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.

7.2 On completion of the reaction, the determination can be restarted by adding D-glucose and/or D-fructose (qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.

7.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.

When analyzing solid samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.

7.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample **and** assay control solution in the same assay. The recovery can then be calculated from the absorbance differences measured.

7.5 Possible losses during the determination can be recognized by carrying out recovery tests: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.

8. Reagent hazard

The reagents used in the determination of D-glucose and D-fructose are not hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulation 67/ 548/EEC and subsequent alteration, supplementation and adaptation guidelines. However, the general safety measures that apply to all chemical substances should be adhered to.

After use, the reagents can be disposed of with laboratory waste, but local regulations must always be observed. Packaging material can be disposed of in waste destined for recycling.

9. General information on sample preparation

In carrying out the assay:
Use **clear, colorless and practically neutral liquid samples** directly, or after dilution according to the dilution table, and of a volume up to 2.000 ml;
Filter **turbid solutions**;
Degas **samples containing carbon dioxide** (e.g. by filtration);
Adjust **acid samples** to approx. pH 8 by adding sodium or potassium hydroxide solution;
Adjust **acid and weakly colored samples** to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min;
Measure **"colored" samples** (if necessary adjusted to pH 8) against a sample blank (= buffer or redist. water + sample), adjust the photometer to 0.000 with the blank in the beam;
Treat **"strongly colored" samples** that are used undiluted or with a higher sample volume with polyvinylpolypyrrolidone (PVPP) or with polyamide, e.g. 1 g/100 ml;
Crush or homogenize **solid or semi-solid samples,** extract with water or

dissolve in water and filter if necessary; resp. remove turbidities or dyestuffs by Carrez clarification;
Deproteinize **samples containing protein** with Carrez reagents;
Extract **samples containing fat** with hot water (extraction temperature should be above the melting point of the fat involved). Cool to allow the fat to separate, make up to the mark, place the volumetric flask in an ice bath for 15 min and filter; alternatively clarify with Carrez-solutions after the extraction with hot water.

Carrez clarification:

Pipette the liquid sample into a 100 ml volumetric flask containing approx. 60 ml redist. water, or weigh sufficient quantity of the sample into a 100 ml volumetric flask and add approx. 60 ml redist. water. Subsequently, carefully add 5 ml Carrez-I-solution (potassium hexacyanoferrate(II) (ferrocyanide), 85 mM = 3.60 g $K_4[Fe(CN)_6] \times 3\ H_2O$/100 ml) and 5 ml Carrez-II-solution (zinc sulfate, 250 mM = 7.20 g $ZnSO_4 \times 7\ H_2O$/100 ml). Adjust to pH 7.5-8.5 with sodium hydroxide (0.1 M; e.g. 10 ml). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.

Samples containing protein should only be deproteinized with perchloric acid or with trichloroacetic acid in the absence of sucrose and maltose as these disaccharides are fully or partically hydrolized with the release of D-glucose. The Carrez clarification is recommended for normal use.

10. Application examples

Determination of D-glucose and D-fructose in fruit juices and similar beverages
Filter turbid juices (alternatively, clarify with Carrez reagents). Dilute the filtrate or clarified fruit juice until the D-glucose + D-fructose concentration is approx. 0.1-1.0 g/l. Decolorization of colored fruit juice is usually not required. Strongly colored juices, which are used undiluted for the assay, are decolorized as follows: add approx. 0.1 g of polyamide powder, gelatine or polyvinylpolypyrrolidone (PVPP) to approx. 10 ml of juice, stir for 1 min, and filter. Use the clear solution (which may still be slightly colored) for the assay.

Determination of D-glucose and D-fructose in wine (Ref. A 2)
Proceed as described for fruit juices. Red wine may also be used directly for the assay without further decolorization.

Fast method: Determination of D-glucose + D-fructose (without differentiation) in white wine with $>$ 5 g total sugar
Dissolve contents of bottle 1 of the Test-Combination with 80 ml redist. water. Add contents of the bottles 2 and 3 and mix carefully.
The solution is stable for 8h at 20-25°C, or for 3 days at 2-8°C.
Place 3.000 ml of the reaction mixture, brought to 20-25°C, into a cuvette (e.g. with a dispenser) and measure absorbance A_1. Start reaction by addition of 0.100 ml of the sample, that has previously been diluted according to the dilution table. Mix and after the end of the reaction (approx. 10-15 min) read absorbance A_2. Calculate absorbance difference $(A_2\text{-}A_1) = \Delta A$.

Calculate concentration of D-glucose + D-fructose:
$c = \Delta A \times 1.596 \times F$ (at Hg 365 nm) [g D-glucose + D-fructose/l sample]
$c = \Delta A \times 0.9037 \times F$ (at Hg 334 nm) [g D-glucose + D-fructose/l sample]
$c = \Delta A \times 0.8865 \times F$ (at 340 nm) [g D-glucose + D-fructose/l sample]
(F = dilution factor)

Determination of D-glucose and D-fructose in beer
To remove the carbonic acid, filter the sample or stir approx. 5-10 ml of beer in a beaker for approx. 30 s with a glass rod. Use the largely CO_2-free sample of beer directly for the assay.

Determination of D-glucose and D-fructose in preserves, specifically dietetic jam, and other vegetable and fruit products
Homogenize about 10 g of sample in a mixer. Accurately weigh approx. 0.5 g of the sample into a 100 ml volumetric flask, mix with water, make up to the mark, mix, and filter. Discard the first 5 ml of filtrate. Use the clear undiluted filtrate directly for the assay (0.100-2.000 ml).

Determination of D-glucose and D-fructose in honey
Stir honey thoroughly with a spatula. Transfer approx. 5-10 g of viscous (or crystalline) honey to a beaker and heat for 15 min at approx. 60°C, stirring ocassionally with the spatula (there is no need to heat liquid honey). Allow to cool. Pour approx. 1 g of the liquid sample, accurately weighed, into a 100 ml volumetric flask, dissolve at first with only a small portion of water, and then dilute to the mark and mix. Prepare a 1:10 (1 + 9) dilution of the 1% honey solution. Use 0.100 ml for the assay.

Determination of D-glucose and D-fructose in desserts and ice-cream
Accurately weigh approx. 1 g of sample into a 100 ml volumetric flask, add about 60 ml water and incubate for 15 min at approx. 70°C; shake from time to time. For clarification, add one after the other and mix after each addition: 5 ml Carrez-I-solution (3.60 g potassium hexacyanoferrate(II), $K_4[Fe(CN)_6] \times 3\ H_2O$/100 ml), 5 ml Carrez-II-solution (7.20 g zinc sulfate, $ZnSO_4 \times 7\ H_2O$/100 ml) and 10 ml NaOH (0.1 M). Adjust to 20-25°C, fill up to the mark with water, mix and filter. Use the clear, possibly slightly opalescent solution for the assay, diluted according to the dilution table.

11. Special preparation of sample for the determination of D-fructose in the presence of a large excess of D-glucose

The precision of the D-fructose determination is impaired if the ratio of D-glucose to D-fructose is higher than e.g. 10:1. In this case, the D-glucose should be as much as possible removed. In the presence of glucose oxidase (GOD) and oxygen from the air, D-glucose is oxidized to D-gluconate:

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{D-gluconate} + H_2O_2$$

Hydrogen peroxide is destroyed by catalase:

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

Reagents
Glucose oxidase (GOD) from Aspergillus niger, 200 U/mg (25°C; D-glucose as substrate); amylase and β-fructosidase $<$ 0.01% each
Catalase
Triethanolamine hydrochloride
$MgSO_4 \times 7\ H_2O$
NaOH, 4 M

Preparation of solutions for 10 determinations
Enzyme solution: Dissolve 5 mg (approx. 1000 U) GOD in 0.750 ml redist. water, add 325 KU catalase (from bovine liver, 25°C; H_2O_2 as substrate), and mix.

Buffer solution: Dissolve 5.6 g triethanolamine hydrochloride and 0.1 g $MgSO_4 \times 7\ H_2O$ in 80 ml redist. H_2O adjust to pH 7.6 with NaOH (4 M), and make up to 100 ml with redist. water.

Stability of solutions
The enzyme solution must be prepared freshly daily.
The buffer solution is stable for 4 weeks when stored at 2-8°C.

Performance of D-glucose oxidation

Pipette into a 10 ml volumetric flask	
buffer solution	2.000 ml
sample solution (up to approx. 0.5% D-glucose)	5.000 ml
enzyme solution	0.100 ml
Pass a current of air (O_2) through the mixture for 1 h; during the oxidation process **check the pH with indicator paper and, if necessary, neutralize the formed acid with NaOH.**	

To inactivate the enzymes GOD and catalase, keep the volumetric flask in a boiling water-bath for 15 min, allow to cool, and fill up to the mark with water. Mix and filter, if necessary. Use the clear solution for the determination of D-fructose. In a parallel assay, determine the residual D-glucose and consider in the calculation as usual.

12. Further applications

The method may also be used in the examination of pharmaceuticals (Ref. A 3.6), cosmetics (Ref. A 3.10), paper (Ref. D 2.2) and tobacco (Ref. C 3.7). Carry out sample preparation and assay as described for analysis of foodstuffs.

The method may also be used, for example, in research when analyzing biological samples. For details of sampling, treatment and stability of the sample see Ref. A 1.

Determination of D-glucose and D-fructose in fermentation samples and cell culture media
Place the sample (after centrifugation, if necessary) in a water-bath at 80°C for 15 min to stop the enzymatic reactions. Centrifuge and use the supernatant (diluted according the dilution table, if necessary) for the assay. (Alternatively, deproteinization can be carried out with perchloric acid, however only in the absence of disaccharides, or with Carrez-solutions. See the above-mentioned examples.)

Homogenize gelatinous agar media with water and treat further as described.

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D-Glucose assay control solution (Bottle 4)

Concentration*: see bottle label

D-Glucose assay control solution is a stabilized aqueous solution of D-glucose. It serves as assay control solution for the enzymatic analysis of D-glucose in foodstuffs and other materials.

Application:

1. Addition of D-glucose assay control solution to the assay mixture:
Instead of sample solution the assay control solution is used for the assay.

2. Restart of the reaction, quantitatively:
After completion of the reaction with sample solution and measuring of A_3, add 0.050 ml assay control solution to the assay mixture. Read absorbance A_4 after the end of the reaction (approx. 15 min). Calculate the concentration from the difference of (A_4-A_3) according to the general equation for calculating the concentration. The altered total volume must be taken into account. Because of the dilution of the assay mixture by addition of the assay control solution, the result differs insignificantly from the data stated on the bottle label.

*Stated as anhydrous D-glucose

3. Internal standard:
The assay control solution can be used as an internal standard in order to check the determination of D-glucose for correct performance (gross errors) and to see whether the sample solution is free from interfering substances:

Pipette into cuvettes	Blank	Sample	Standard	Sample + Standard
solution 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml
sample solution	-	0.100 ml	-	0.050 ml
assay control sln.	-	-	0.100 ml	0.050 ml
redist. water	2.000 ml	1.900 ml	1.900 ml	1.900 ml
Mix, and read absorbances of the solutions (A_1) after approx. 3 min. Continue as described in the pipetting scheme under "Procedure". Follow the instructions given under "Instructions for performance of assay" and the footnotes.				

The recovery of the standard is calculated according to the following formula:

$$\text{recovery} = \frac{2 \times \Delta A_{\text{sample + standard}} - \Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 100\ [\%]$$

4. Note
An assay control solution of D-fructose cannot be prepared and delivered with the Test-Combination D-Glucose/D-Fructose because an aqueous solution of D-fructose is not stable enough.

For further information see instructions for

Test-Combination	**D-Glucose**	**Cat. No. 10 716 251 035**
Test-Combination	**Maltose/Sucrose/ D-Glucose**	**Cat. No. 11 113 950 035**
Test-Combination	**Sucrose/D-Glucose**	**Cat. No. 10 139 041 035**
Test-Combination	**Sucrose/D-Glucose/ D-Fructose**	**Cat. No. 10 716 260 035**
Test-Combination	**D-Sorbitol/Xylitol**	**Cat. No. 10 670 057 035**
Test-Combination	**Starch**	**Cat. No. 10 207 748 035**

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