D–Sorbitol/Xylitol

Colorimetric method

for the determination of D-sorbitol and xylitol[1] in foodstuffs and other materials

Cat. No. 10 670 057 035

Test-Combination for 3 × approx. 12 determinations

Principle (Ref. 1)

D-Sorbitol and xylitol are oxidized by nicotinamide-adenine dinucleotide (NAD) to D-fructose or D-xylulose, respectively, in the presence of the enzyme sorbitol dehydrogenase (SDH, also called polyol dehydrogenase) with the formation of reduced nicotinamide-adenine dinucleotide (NADH) (1a, 1b).

(1a) D-Sorbitol + NAD$^+$ $\xrightleftharpoons{\text{SDH}}$ D-Fructose + NADH + H$^+$

(1b) Xylitol + NAD$^+$ $\xrightleftharpoons{\text{SDH}}$ D-Xylulose + NADH + H$^+$
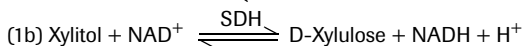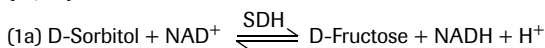
Under the assay conditions, the equilibrium of the reactions (1a, 1b) lies on the side of NAD and D-sorbitol or xylitol, respectively. However, they are favourably displaced as the formed NADH is removed in a subsequent reaction in which NADH reduces iodonitrotetrazolium chloride (INT) to a formazan in the presence of diaphorase (2).

(2) NADH + INT + H$^+$ $\xrightarrow{\text{diaphorase}}$ NAD$^+$ + formazan
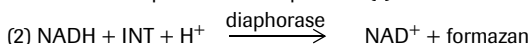
The absorbance of the formazan is measured at its maximum at 492 nm.

The Test-Combination contains

1. Bottle 1 with approx. 25 ml solution, consisting of:
   potassium phosphate/triethanolamine buffer, pH approx. 8.6; Triton X-100 (trademark of Rohm & Haas, Philadelphia, USA)
2. Three bottles 2 with each approx. 35 mg of lyophilizate, consisting of: diaphorase, approx. 4 U; NAD, approx. 28 mg
3. Bottle 3 with iodonitrotetrazolium chloride solution, approx. 2.5 ml
4. Three bottles 4, with lyophilizate SDH, approx. 25 U, each
5. Bottle 5 with D-sorbitol assay control solution for assay control purposes (measurement of the assay control solution is not necessary for calculating the results.) Use the assay control solution undiluted. (Expiry date: see pack label)

Preparation of solutions

1. Use contents of bottle 1 undiluted.
2. Dissolve contents of one bottle 2 with 2.5 ml redist. water.
3. Dilute contents of bottle 3 with 6 ml redist. water.
4. Dissolve contents of one bottle 4 with 0.6 ml redist. water. Slight opalescence of the solution does not interfere with the assay.

Stability of reagents

Solution 1 is stable at 2-8°C (see pack label).
   Bring solution 1 to 20-25°C before use.
The contents of the bottles 2 are stable at 2-8°C (see pack label).
   Solution 2 is stable for 1 week at 2-8°C.
   Bring solution 2 to 20-25°C before use.
The contents of bottle 3 are stable at 2-8°C (see pack label).
   Solution 3 is stable for 3 months at 2-8°C, or for 1 month at 20-25°C stored in the dark.
   Bring solution 3 to 20-25°C before use.
The contents of the bottles 4 are stable at 2-8°C (see pack label).
   Solution 4 is stable for two weeks at 2-8°C, and for 4 weeks at −15 to −25°C.

Procedure

Wavelength: (Hg) 492 nm
Glass cuvette[2]:   1.00 cm ligth path
Temperature: 20-25°C
Final volume: 3.050 ml
Read against air (without a cuvette in the light path) against water or blank
Sample solution: 0.4-10 μg D-sorbitol and/or xylitol/assay[3]
                 (in 0.100-2.000 ml sample volume)

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1   If D–sorbitol and xylitol are present, the sum of both sugar alcohols is determined.
2   If desired, disposable cuvettes may be used instead of glass cuvettes.
3   See instructions for performance of assay
4   For series analysis, a suitable stock solution may be prepared by mixing solutions 1, 2 and 3. This stock solution is stable for 1 h when stored in the dark at 20-25°C. Use 1.000 ml for the assay.
5   **INT is sensitive to light. After addition of solution 3, do not store the cuvettes in the light.**

Pipette into cuvettes	Blank	Sample
solution 1[4]	0.600 ml	0.600 ml
solution 2[4]	0.200 ml	0.200 ml
solution 3[4, 5]	0.200 ml	0.200 ml
sample solution*	-	0.100 ml
redist. water	2.000 ml	1.900 ml

Mix**, after 2 min read absorbances of the solutions (A$_1$). Repeat the measurement after 2 min.
If a change in absorbance greater than 0.010 is observed, the sample must be treated according to pt. 7 (removal of reducing substances). However, if the change in absorbance is less than 0.010, such pre-treatment is not necessary, providing the reaction is started immediately after the previous measurement by adding of:

solution 4	0.050 ml	0.050 ml

Mix**, wait until reaction has stopped (approx. 30 min), and read absorbances of the solutions (A$_2$).
If the reaction has not stopped after 30 min, continue to read the absorbances at 5 min intervals until the absorbance increases constantly for 5 min.

*   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)

If the absorbance A$_2$ increases constantly, extrapolate the absorbance to the time of the addition of solution 4 (SDH).

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.

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

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 calculation 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 INT-formazan at 492 nm
    = 19.9 [l × mmol^{-1} × cm^{-1}]

It follows for D-sorbitol:

$$c = \frac{3.050 \times 182.17}{19.9 \times 1.00 \times 0.100 \times 1000} \times \Delta A = 0.2792 \times \Delta A_{\text{D-sorbitol}} \ [\text{g D-sorbitol/l sample solution}]$$

for xylitol:

$$c = \frac{3.050 \times 152.15}{19.9 \times 1.00 \times 0.100 \times 1000} \times \Delta A = 0.2332 \times \Delta A_{\text{xylitol}} \ [\text{g xylitol/l sample solution}]$$

This calculation is only valid in the case of the presence of either D-sorbitol or xylitol in the sample / the assay.

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

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

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

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

1. Instructions for performance of assay

The D-sorbitol content or xylitol content, respectively, present in the assay has to be between 0.4 and 10 μg. In order to obtain a sufficient absorbance difference the sample solution is diluted to yield a concentration between 0.01 and 0.10 g/l.

Dilution table

Estimated amount of D-sorbitol or xylitol per liter	Dilution with water	Dilution factor F
< 0.10 g	-	1
0.10-1.0 g	1 + 9	10
1.0-10 g	1 + 99	100
> 10 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

2.1 For series analysis, a suitable stock solution may be prepared by mixing solutions 1, 2 and 3. This stock solution is stable for 1 h when stored at 20-25°C in the dark. Use 1.000 ml for each assay.

2.2 The reaction system is sensitive to light (daylight or artificial light) after the addition of INT (solution 3 or reaction mixture). The incubation has to be done in the dark:

a) if incubating in the photometer, close cuvette compartment of the photometer and block out light.

b) Cover the cuvettes or store in a darkened cupboard.

3. Specificity (Ref. 1)

Besides D-sorbitol and xylitol, sorbitol dehydrogenase also oxidizes other polyols, such as iditol, allitol, ribitol although with lower velocity. Other polyalcohols such as mannitol, arabitol, dulcitol do not react.

Under the above-mentioned assay conditions, glycerol is practically not oxidized (less than 0.2 % conversion with 100 μg of glycerol/assay. With a higher enzyme activity a measurable reaction can be observed; see also Ref. 1).

In the analysis of commercial D-sorbitol and xylitol, results of approx. 100 % have to be expected.

4. Sensitivity and detection limit (Ref. 1)

The smallest differentiating absorbance for the procedure is 0.005 absorbance units. This corresponds to a maximum sample volume v = 2.000 ml of a D-sorbitol, resp. xylitol concentration of approx. 0.1 mg/l sample solution (if v = 0.100 ml, this corresponds to approx. 1.2 mg/l sample solution).

The detection limit of approx. 0.2 mg/l is derived from the absorbance difference of 0.015 and a maximum sample volume v = 2.000 ml.

5. Linearity

Linearity of the determination exists from 0.4 μg D-sorbitol, resp. xylitol/assay (0.2 mg D-sorbitol, resp. xylitol/l sample solution; sample volume v = 2.000 ml) to 10 μg D-sorbitol, resp. xylitol/assay (0.1 g D-sorbitol, resp. xylitol/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 this corresponds to a D-sorbitol or xylitol concentration of 1.5-3 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 0.015-0.03 g/100 g can be expected.)

The following data have been published in the literature:

D-Sorbitol solutions:

10 μg/assay	n = 15	CV = 1.6 %
30 μg/assay	n = 15	CV = 0.6 %
70 μg/assay	n = 15	CV = 1.0 %

Xylitol solutions:

4 μg/assay	n = 15	CV = 1.62 %
8 μg/assay	n = 15	CV = 1.62 % (Ref. 1.3)

Analysis of bakery goods (Ref. 2.1):

x = 4.56 g/100 g n = 10 s = ± 0.149 g/100 g CV = 3.27 %
x = 18.76 g/100 g n = 10 s = ± 0.283 g/100 g CV = 1.51 %
x = 4.66 g/100 g n = 19 r = 0.22 g/100 g R = 0.35 g/100 g
  r_{rel} = 4.72 % R_{rel} = 7.51 %
x = 19.51 g/100 g n = 17 r = 0.64 g/100 g R = 1.28 g/100 g
  r_{rel} = 3.28 % R_{rel} = 6.56 %

7. Interference

High concentrations of reducing substances e.g. L-ascorbic acid in fruit juices or SO_2 in fruit (jam) interfere with the assay since they react with INT, thus causing a "creep reaction". Therefore, quantities larger than 5 μg of L-ascorbic acid or SO_2/assay should be removed by treating the sample with H_2O_2 and alkali.

For this purpose weigh (or pipette) sample, diluted, if necessary, containing approx. up to 5 mg D-sorbitol or xylitol, respectively, into a 50 ml volumetric flask. Fill up to approx. 40 ml with water, add 1 ml KOH (2 M) and 0.01 ml H_2O_2 (30%; w/v). Incubate solution for 10 min at approx. 70°C. After cooling to 20-25°C adjust to pH 7-8 with sulfuric acid (1 M). Fill up to the mark with water, mix, filter and use the solution for the assay.

D-Fructose up to 1 mg/assay and butylene glycol up to 500 μg/assay do not interfere with the assay (reaction time: 60 min).

Recognizing interference during the assay procedure

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

8.2 On completion of the reaction, the determination can be restarted by adding D-sorbitol or xylitol (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.

8.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carring 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.

8.4 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.

8.5 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.

9. Reagent hazard

The reagents used in the determination of D-sorbitol/xylitol contain hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulations 67/548 and 99/45 and subsequent alteration, supplementation and adaptation guidelines. Please refer to the safety date sheet or the labels of the affected vials for further information.

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10. General information on sample preparation

In carrying out the assay:

Use **clear, colorless or colored 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;

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 perchlorid acid; alternatively clarify 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, fill 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 which contains 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.

11. Application examples

Determination of D-sorbitol resp. xylitol in fruit juices

Pipette 5.0 ml of black currant juice, resp. 1.0 ml of apple juice (filter turbid juice before) into a 50 ml volumetric flask. Add successively 30 ml redist. water, 1 ml KOH (2 M) and 0.05 ml (in the case of apple juice 0.02 ml) hydrogen peroxide solution (30%; w/v), mix and incubate for 15 min at 20-25°C. Then adjust pH to approx. 8 by the addition of sulfuric acid (1 M). Add 2600 U catalase[6], mix and incubate for 20 min (10 min in the case of apple juice), fill up the volumetric flask to the mark with water, mix and filter. Use the filtrate for the assay.

Determination of D-sorbitol or xylitol in diabetic honey

Accurately weigh approx. 5 g of honey into a 100 ml volumetric flask and add approx. 70 ml water. Incubate for 10 min at approx. 70°C. Allow to cool and fill up to the mark with water. Dilute solution according to the dilution table and use it for the assay.

Determination of D-sorbitol or xylitol in diabetic jam

Homogenize approx. 10 g of jam for 2 min in an electric mixer (homogenizer). Accurately weigh approx. 1 g of the homogeneous material into a 100 ml volumetric flask, add approx. 50 ml water and incubate for 5 min at approx. 60°C. Allow to cool to 20-25°C, fill up to the mark with water. Mix and filter. Dilute with water according to the dilution table, if necessary, and use it for the assay.

Determination of D-sorbitol or xylitol in ice-cream

Accurately weigh approx. 1 g of sample into a 100 ml volumetric flask, add approx. 60 ml redist. water and incubate for 15 min at approx. 70°C; shake from time to time. For clarification, add 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), mix after each addition, adjust to 20-25°C, fill up to the mark with water, and filter. Use the clear, possibly slightly turbid solution for the assay, diluted, if necessary.

Determination of D-sorbitol or xylitol in marzipan and sweets

Accurately weigh approx. 5 g of the homogenized and minced sample into a 100 ml volumetric flask, add. approx. 60 ml water and keep the volumetric flask for approx. 15 min in a water-bath at approx. 60°C whilst occasionally shaking. After cooling to 20-25°C fill up to the mark with water. Mix and filter, centrifuge, if necessary. Dilute the clear solution, if necessary, and use it for the assay.

Determination of D-sorbitol and xylitol in protein–containing samples

Deproteinize protein-containing sample solutions with ice-cold perchloric acid (1 M) in a ratio of 1:2 (1 + 1), centrifuge and neutralize the supernatant with KOH (1 M).

Determination of D-sorbitol or xylitol in diabetic chocolate

Accurately weigh approx. 3 g ground chocolate into a 100 ml volumetric flask, add 70 ml water and heat to approx. 70°C in water-bath while shaking. Allow suspension to cool to 20-25°C and fill up to the mark with water. To obtain separation of fat, place the volumetric flask for 20 min in the refrigerator. Centrifuge solution (for 5 min at approx. 5000 rpm). Dilute the clear supernatant solution, if necessary, and use it for the assay.

Determination of D-sorbitol or xylitol in diabetic bakery products

Mince and homogenize approx. 10 g of bakery products in a mortar or electric mixer. Accurately weigh approx. 3 g of the sample into a 100 ml volumetric flask and add approx. 70 ml water. Place the volumetric flask for 15 min into a water-bath at approx. 70°C. After cooling to 20-25°C fill up to the mark with water. Keep for 20 min in a refrigerator for separation of fat. Centrifuge sample solution for approx. 5 min (at approx. 5000 rpm). Dilute the clear, or slightly turbid supernatant solution according to the dilution table and use it for the assay.

Determination of D-sorbitol or xylitol in diabetic pudding mix

Accurately weigh contents of one bag (approx. 10-15 g) into a 1 l Erlenmeyer flask and add approx. 500 ml water. Bring mixture to boil and let it simmer for 2 min; allow to cool, transfer quantitatively with water into a 1 l volumetric flask and fill up to the mark. After mixing, centrifuge part of the mixture (for approx. 5 min at approx. 5000 rpm). Dilute the clear or slightly turbid supernatant solution according to the dilution table and use it for the assay.

12. Further applications

The method may also be used in the examination of pharmaceuticals, cosmetics, paper and in research when analyzing biological samples. For details of sampling, treatment and stability of the sample see Ref. 1.

Determination of D-sorbitol or xylitol 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 enzymatic reactions. Centrifuge and use the supernatant (diluted according to the dilution table, if necessary) for the assay.

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

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[6] from bovine liver (25°C, H_2O_2 as substrate)

References

1.1 Bergmeyer, H. U., Gruber, W. & Gutmann, I. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H. U., Hrsg.) 3. Aufl., Bd. 2, S. 1368-1371, Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 2nd ed., vol. 3, p. 1323-1330, Verlag Chemie, Weinheim/Academic Press, Inc., New York, London

1.2 Bässler, K.H. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, S. 1425-1428, Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed., vol. 3, p. 1323-1330, Verlag Chemie, Weinheim/ Academic Press, Inc., New York, London

1.3 Beutler, H. O. & Becker. J. (1977) Enzymatische Bestimmung von D-Sorbit und Xylit in Lebensmitteln, Deutsche Lebensmittel-Rundschau **6**, 182-187

1.4 Beutler, H.-O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 3rd ed., vol. VI, pp. 484-490, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

2.1 Beutler, H.-O. & Dresselhaus-Schroebler, M. (1993) Ringversuche zur Bestimmung von D-Sorbit in diätetischen Backwaren, Deutsche Lebensmittel-Rundschau **89**, 349-351

2.2 Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Untersuchung von Lebensmitteln: Bestimmung von D-Sorbit in Feinen Backwaren, 18.00-14 (Mai 1994)

2.3 International Federation of Fruit Juice Producers (IFU, Methods of Analysis, no. 62-1995); contained in "Code of Practice for Evaluation of Fruit and Vegetable Juices" (1996) edited by Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.).

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D-Sorbitol assay control solution (Bottle 5)

Concentration: see bottle label

D-Sorbitol assay control solution is a stabilized aqueous solution of D-sorbitol. It serves as an assay control solution for the enzymatic determination of D-sorbitol in foodstuffs and other materials.

Application:

1. Addition of D-sorbitol 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_2, add 0.050 ml assay control solution to the assay mixture. Read absorbance A_3 after the end of the reaction (approx. 30 min). Calculate the concentration from the difference of (A_3-A_2) 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.

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

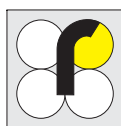| Pipette into cuvettes	Blank	Sample	Standard	Sample + Standard
solution 1	0.600 ml	0.600 ml	0.600 ml	0.600 ml
solution 2	0.200 ml	0.200 ml	0.200 ml	0.200 ml
solution 3	0.200 ml	0.200 ml	0.200 ml	0.200 ml
sample solution	-	0.100 ml	-	0.050 ml
assay control sln.	-	-	0.100 ml	0.050 ml
redist. water	2.000 ml	1.900 ml	1.900 ml	1.900 ml

Mix, and read absorbances of the solutions (A_1) after approx. 2 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:

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

For further information see instructions of

Test-Combination	**D-Glucose**	**Cat. No. 10 716 251 035**
Test-Combination	**D-Glucose/D-Fructose**	**Cat. No. 10 139 106 035**
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	**Starch**	**Cat. No. 10 207 748 035**

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