UV method for approx. 32 assays

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

The method is contained in the Dutch, German, Swiss food laws. Recommended e. g. by AIJN, MEBAK. Standardized by DIN, NEN.

Principle

Starch + (n-1) H_2O —— Amyloglucosidase (AGS) ⟶ n D-glucose
D-Glucose + ATP —— Hexokinase ⟶ G-6-P + ADP
G-6-P + $NADP^+$ —— G6P-DH ⟶ 6-PG + NADPH + H^+

Ref.: Beutler, H.-O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., vol. VI, pp. 2-10; Verlag Chemie Weinheim, Deerfield / Florida, Basel.

Assay performance

Wavelength: 340 nm (NADPH), $\varepsilon = 6.3\ l \times mmol^{-1} \times cm^{-1}$
Light path: 1.00 cm (glass or plastic cuvettes)
Temperature: +55 to +60°C (incubation)
+20 to +25°C (measurement)
Assay volume: 2.320 ml
Measurement: against air or against water
Sample solution: 1.2 to 70 µg starch in 0.100 to 1.000 ml sample solution, resp. 0.100 to 0.200 ml when analyzing DMSO containing solutions.

Reagents

AGS: Lyophilizate with citrate buffer, pH approx. 4.6, approx. 98 U amyloglucosidase (for stability see pack label). *Dissolve contents of bottle # AGS with 7 ml redist. water.* The solution is stable for 6 weeks at +2 to +8°C, resp. for 3 months at -15 to -25°C.

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. for 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 material starch.

The reagents for the determination of starch 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:	**Standard blank**[1,2]	**Standard assay**[1,2]	**Sample blank**[2,3]	**Sample assay**[2,3]	**Reagent Blank**[4]	**Sample assay**[4]
Citrate buffer pH 4.6, AGS solution # AGS	-	0.200 ml	-	0.200 ml	0.200 ml	0.200 ml
Sample solution[5] **(e. g. 0.03 to 0.7 g starch/l)**	**-**	**-**	**0.100 ml**	**0.100 ml**	**-**	**0.100 ml**
Standard solution[5] (e. g. 0.7 g starch/l)	0.100 ml	0.100 ml	-	-	-	-
Mix e. g. by gentle swirling of the cuvette. Incubate the closed cuvette at +55 to +60 °C for 15 min. Bring to room temperature again and add:						
Tea buffer pH 7.6, NADP, ATP solution # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
Redist. Water	1.200 ml	1.000 ml	1.200 ml	1.000 ml	1.100 ml	1.000 ml
Mix[6]**, 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[6]**, after approx. 10 to 15 min read the absorbances (A_2). Repeat absorbance reading after another 2 min**[7]**.**						

Notes

1 Run a "standard" to see "accidents" in sample preparation and performance of assays. The measurement of the standard is not necessary for calculating results.
2 Pipeting scheme after solubilisation of starch with DMSO/HCl.
3 This assay together with the blank is a single determination. 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. (When analyzing DMSO containing solutions the maximum sample volume is 0.200 ml.)
4 After sample preparation with hydrochloric acid. (The sample volume can be increased up to 1.000 ml. See also note 3.)
5 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
6 e. g. with a plastic spatula, or after closing the cuvette with Parafilm (trademark of American Can Co., Greenwich Ct., USA).
7 The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read them until they increase constantly over 2 min. Extrapolate absorbances to the time of the addition of HK/G6P-DH (suspension # 2).

Calculation

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

C = (V x MW x ΔA) / (ε x d x v x 1000) [g starch/l sample solution]

c = (2.320 x 162.1 x ΔA) / (6.3 x 1.00 x 0.100 x 1000) = **0.5969 x Δ A [g starch/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 sample preparation, calculate the content from the amount weighed:

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

Sample preparation

1. *Solubilise starch with DMSO and HCl*: Accurately weigh 100 mg to 1 g of the homogenized sample (containing approx. 70 mg starch) into a 100 ml-Erlenmeyer flask; add 20 ml DMSO and 5 ml HCl (8 M) in the case of fat containing samples, resp. 5 ml HCl (8 M) and 20 ml DMSO in the case of fat-free samples; close the flask and incubate in a shaking water-bath at 55-60°C for 30 (normally) to 60 min (in the case of 'very hard' materials), or under heatable magnetic stirring; cool to room temperature; add approx. 50 ml redist. water; adjust pH to 4 to 5 (not higher!) by adding NaOH (5 M); transfer the material quantitatively into a 100 ml-volumetric flask (rinse with water), fill up to the mark and mix; let stand for a few minutes and take the sample solution from the top of the solution by means of a piston-type pipette.
2. *Solubilise starch partially with HCl*: Accurately weigh 100 mg to 1 g of the homogenized sample (containing approx. 70 mg starch) into a centrifuge tube; wash 3 times with a ethanol/water mixture (e. g. 40 %; v/v): stir at room temperature for approx. 20 min, centrifuge, remove the supernatant; add 10 ml HCl (approx. 10 %; m/m), stir at +60 °C in a water bath for 60 min; transfer the material quantitatively into a 100 ml beaker, rinse the centrifuge tube with redist. water and transfer to the beaker again (100% of the sample must be transferred); adjust pH to 4 to 5 (not higher!) by adding NaOH (5 M); transfer the material quantitatively into a 100 ml-volumetric flask (rinse the beaker with water and transfer again), fill up to the mark and mix; let stand for a few minutes and take the sample solution from the top of the solution by means of a piston-type pipette.
   Note: Samples containing water are treated with ethanol: add 10 ml ethanol (96 %; v/v) to approx. 1 g sample, stir and centrifuge; decant the ethanol and continue as described above.
3. *Determination of "glucose syrup" e. g. in fruit juices*: Accurately weigh 100 mg to 1 g of the homogenized sample (containing approx. 100 mg D-glucose + "glucose syrup") into a volumetric flask; fill up to the mark with redist. water, mix and filter. Perform the analysis with sample blank assay and sample assay (incubate 30 min at +20 to +25°C).
4. *Determination of dextrins in beer*: Use the sample directly for the assay: perform analysis with sample blank and sample assay.

Assay characteristics

1. *Specificity:* AGS hydrolyzes α-1,4- and α-1,6-glucosidic bonds independent on the molecular weight (amylose, amylopectin, glycogen, dextrin, maltose, maltotriose, etc.). A limited differentiation is only possible during sample preparation by washing with ethanol/water mixtures. In the analysis of pure starch results of 99 % calculated on the dry mass have to expected.
2. *Sensitivity:* 3 mg starch/l (ΔA = 0.010; v = 0.200 ml; V = 2.320 ml)
3. *Detection limit*: 6 mg starch/l (ΔA = 0.020; v = 0.200 ml; V = 2.320 ml)
4. *Linearity*: from 1.2 µg starch/assay (v = 0.200 ml; V = 2.320 ml)
   to 70 µg starch/assay (v = 0.100 ml; V = 2.320 ml)
5. *Precision:* ΔA = +/- 0.010 to 0.015 absorbance units
   CV = approx. 1 to 2 %

Pork sausage:	x = 1.3 g/100 g	r = 0.170 g/100 g	s(r) = +/-0.060 g/100 g
		R = 0.217 g/100 g	s(R) = +/-0.077 g/100 g
Children's rusks:	x = 43.5 g/100 g	r = 2.33 g/100 g	s(r) = +/-0.82 g/100 g
		R = 8.42 g/100 g	s(R) = +/-2.97 g/100 g

6. *Technical Information:*

6.1 D-Glucose cannot be calculated from the sample blank because it contains also D-glucose from sucrose, lactose and maltose liberated during sample preparation with DMSO and HCl. A separate sample preparation is necessary.

6.2 If the sample contains maltose, the sample has to be washed with ethanol/water mixtures otherwise a part of maltose will be found in the sample blank and the rest in the sample assay because maltose is only partially hydrolyzed during sample preparation with DMSO/HCl.