UV assay for the determination of native starch in foodstuffs and other sample materials
Test combination for 50 determinations

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

1. Test principle

The enzyme amyloglucoamylase (AGS) cleaves starch to D-glucose:

Starch + (n-1) H_2O $\xrightarrow{AGS}$ n D-glucose

The formed D-glucose is phosphorylated with ATP in the presence of the enzyme hexokinase (HK) to D-glucose-6-phosphate (G-6-P), simultaneously producing ADP:

D-glucose + ATP $\xrightarrow{HK}$ D-glucose-6-phosphate + ADP

In the presence of a glucose-6-phosphate-dehydrogenase (G6P-DH), D-glucose-6-phosphate is oxidized to D-gluconate-6-phosphate:

G-6-P + NAD^+ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-phosphate + NADH + H^+

Nicotinamide-adenine-dinucleotide (NAD) is reduced to NADH. The amount of NADH formed is proportional to the amount of D-glucose formed and is measured at 340 nm.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 2 x 50 mL with buffer, AGS, ATP, NAD
- Reagent 2: 2 x 12.5 mL with buffer, HK, G6P-DH

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- Important! The sample must be processed sequentially. This means that the extraction must not be stopped at any point.
- Weigh 50 - 500 mg of a homogenized sample (maximum total starch content 50 mg) into a 50 mL Erlenmeyer flask.
- Add 5 mL HCl 32 % and stir for 2 - 3 min. Then add 15 mL DMSO, seal the Erlenmeyer flask with parafilm and stir at 60 °C for at least 60 min.
- Cool rapidly to 20 - 25 °C, transfer to a 50 mL volumetric flask and add 5 mL 8 M NaOH.
- Rinse with 0.1 M citrate buffer, pH 4.0, and make up to 50 mL.
- Incubate the sample solution for 15 min at 50 - 55 °C before determination.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 10 - 1000 mg/L

Important: Incubate the sample 15 min at 50 - 55 °C before determination.		
	Reagent blank	**Samples / controls**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 10 min at 20 - 37 °C. Read absorbance A_1, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 10 min at 20 - 37 °C and read absorbance A_2.		

The reagent blank value must be determined once for each run and subtracted from each sample result.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of starch

$$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume + R1}}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df).

$$\mathbf{C_{total\ starch}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.669 \times \Delta A}$$

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 162.14
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

5.1.2. Calculation of the real starch concentration

The result of the E8100 test additionally includes the amounts of maltose, D-glucose, their oligomers and derivates as well as sucrose that might be present in the sample.
It is calculated using the molecular weight of the starch monomers (162.14 g/mol) and is referred to as *total starch*.
To determine the real starch concentration, the sum of sugar (maltose, D-glucose, their oligomers and derivatives as well as sucrose) must be determined using the Enzytec™ *Liquid* Maltose/Sucrose/D-Glucose assay (E8170).
The result is expressed as *total maltose* (342.3 g/mol) and subtracted from *total starch* for differentiation:

$$C_{starch}\ [g/L] = C_{total\ starch\ E8100} - 0.95 \times C_{total\ maltose\ E8170}$$

Make sure that the same dilution factors are used to determine the concentration of total starch and total maltose. If necessary, these must be adjusted according to the individual extractions.

5.2. Calculation of solid samples

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

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend a commercially available starch material with known purity and water content.
The recovery of pure starch control solutions should be within 100 ± 5 % and for extracted starch samples within 100 ± 10 %.

6. Performance data

6.1. Specificity & side activities

Amyloglucosidase hydrolyzes α-1,4- and α-1,6-glycosidic bonds. These occur in starch (amylose and amylopectin) as well as in polysaccharides such as dextrin, glycogen and glucosyl oligosaccharides. Maltose, maltodextrin and glucose show a side activity of over 90 % (sucrose reacted at 52 %). The included hexokinase is specific for D-glucose.
Sample solutions containing sucrose, maltose, free D-glucose and other oligosaccharides must be tested separately with the Enzytec™ *Liquid* Maltose/Sucrose/D-Glucose (E8170). The total maltose content obtained must be subtracted from the total result as described in section *Calculation of results*.

6.2. Interferences

Sulphur dioxide and ascorbic acid interfere from a concentration > 2 g/L and citric acid from a concentration > 50 g/L.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 1000 mg/L total starch, with the recommended measuring range between 10 and 1000 mg/L.
The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11 in buffered aqueous solution. This results in an LoD of 3.0 mg/L. The limit of quantification (LoQ) was determined by a precision profile and is 10.0 mg/L.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an LoD of 0.09 mg/L. Based on ΔA = 0.010, an LoQ of 0.18 mg/L was calculated.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

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