Enzytec^{TM} *Liquid* Starch — Art. No. E8100

Version 2 / 2026-03-31

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 (36 – 46 °F)

This test was evaluated using selected samples of the following matrices: Beer, infant formula, pet food, and cheese. In addition, samples from the following matrices were tested for the method comparison: starch (potato, wheat, corn), flour (wheat, corn), and pudding powder.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

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

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

The enzyme hexokinase phosphorylates D-glucose with adenosine 5'-triphosphate (ATP) to form D-glucose-6-phosphate (G-6-P), while simultaneously producing adenosine 5'-diphosphate (ADP):

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

In the presence of glucose-6-phosphate dehydrogenase (G6PD), G-6-P is oxidized by nicotinamide adenine dinucleotide (NAD) to D-gluconate-6-phosphate, producing NADH:

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

The amount of NADH formed during the reaction is proportional to the amount of D-glucose produced by the hydrolysis of starch. NADH serves as the parameter and is measured based on its absorption 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 and NAD
Reagent 2:	2 x 12.5 mL with buffer, HK and G6P-DH

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 – 25 °C / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

3.1. Notes on 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.
- The starch content of a sample must be ≤ 50 mg. If the starch concentration in the sample is too high, the extraction will be insufficient.
- A sample with unknown starch content must be extracted with at least two weights, e.g. 50 and 500 mg.
- The water content of pure starch samples must be taken into account.
- The sample shall not cool down below 20 – 25 °C (68 – 77 °F) after extraction at 60 °C (140 °F), otherwise starch can precipitate again.

3.2. Solubilization of starch with dimethylsulfoxide (DMSO) and hydrochloric acid (HCl)

For use in a variety of applications, including starch products, flours, baked goods, jams, meatballs, meat products, dairy products, margarine, and animal feed.

- Grind and homogenize solid and semi-solid samples (e.g., in a powder mill or homogenizer).
- Weigh 50 – 500 mg of the homogenized sample into a 50 mL Erlenmeyer flask.
- Add 5 mL HCl 32 % and stir for 2 – 3 minutes. Then add 15 mL DMSO, seal the Erlenmeyer flask with parafilm and stir at 60 °C (140 °F) for at least 60 minutes (use a shaking water bath or a heated magnetic stirrer; no clumping should occur; break up any clumps with a glass rod if necessary).
- Cool rapidly to 20 – 25 °C (68 – 77 °F), transfer to a 50 mL volumetric flask and add 5 mL 8 M NaOH.
- Rinse with 0.112 M citrate buffer (pH 4.0, mixture of 0.112 M citric acid solution and 0.112 M trisodium citrate solution), and make up to 50 mL.
- Filter the solution if necessary (wash the filter paper beforehand with boiling-hot distilled water). Centrifugation is not recommended, as the precipitation of starch may result in lower-than-expected values.
- Incubate the sample solution for 15 min at 50 – 55 °C (122 – 131 °F) before determination.

3.3. Infant formula based on § 64 LFGB L 48.01-05

- Weigh 500 mg of a homogenised sample into a 100 mL Erlenmeyer flask.
- Add 5 mL 8 M HCl and stir for approx. 2 – 3 minutes.
- Add 20 mL DMSO and seal the Erlenmeyer flask with Parafilm; stir for at least 30 minutes at 60 °C (140 °F) in a shaking water bath or on a magnetic heating stirrer.
- Cool down quickly (approx. 3 minutes) to 20 – 25 °C (68 – 77 °F) under running water or short in an ice bath.
- Add 50 mL distilled water and adjust the pH to 4 – 5 with 5 M NaOH.
- Transfer the solution quantitatively to a 200 mL volumetric flask and rinse with distilled water.
- After re-tempering and removing the stirring magnet, fill to the mark, shake well, and filter.
- Discard the first millilitres of the filtrate.
- Use 100 µL sample solution and analyze immediately in the test.

3.4. Cheese according to § 64 LFGB L 03.00-39

- Weigh 1 – 2 g (starch content < 2 g/100 g) or 0.5 g sample (starch content > 2 g/100 g) of a homogenised sample into a 100 mL Erlenmeyer flask.
- Suspend the sample in 20 mL DMSO and add 5 mL HCl 32 %
- Seal the Erlenmeyer flask with Parafilm; stir for at least 30 – 60 minutes at 60 °C (140 °F) in a shaking water bath or on a magnetic heating stirrer. Crush any clumps that may form.

- Cool down quickly (approx. 3 minutes) to 20 – 25 °C (68 – 77 °F) under running water or short in an ice bath.
- Add 50 mL distilled water and adjust the pH to 4 – 5 with 5 M NaOH (approx. 4 mL).
- Transfer the solution quantitatively to a 100 mL volumetric flask and rinse with distilled water.
- After cooling down, fill up to the mark, shake well, leave to stand for a few minutes and filter.
- Discard the first millilitres of the filtrate.
- Use 100 µL sample solution and analyze immediately in the test.

3.5. Further application examples

3.5.1. Beer

- Dissolve 1 mL beer in 5 mL HCl 32 % and 15 mL of DMSO.
- Heat at 60 °C (140 °F) for 120 minutes, cool rapidly to 20 – 25 °C (68 – 77 °F), and add 5 mL 8 M NaOH.
- Transfer the solution to a 50-mL volumetric flask, rinse with 0.112 M citrate buffer, and fill up to the mark.
- Proceed as described in Chapter *3.2 Solubilization of starch with dimethylsulfoxide (DMSO) and hydrochloric acid (HCl).*

3.5.2. Pudding powder & flours

- Dissolve 50 mg of pudding powder or flour in 5 mL HCl 32 % and 15 mL of DMSO.
- Heat at 60 °C (140 °F) for 60 minutes, cool rapidly to 20 – 25 °C (68 – 77 °F), and add 5 mL 8 M NaOH.
- Transfer the solution to a 50-mL volumetric flask, rinse with 0.112 M citrate buffer, and fill up to the mark.
- Proceed as described in Chapter *3.2 Solubilization of starch with dimethylsulfoxide (DMSO) and hydrochloric acid (HCl).*

3.5.3. Jam

- Dissolve 50 mg of jam in 5 mL HCl 32 % and 15 mL of DMSO.
- Heat at 60 °C (140 °F) for 180 minutes, cool rapidly to 20 – 25 °C (68 – 77 °F), and add 5 mL 8 M NaOH.
- Transfer the solution to a 50-mL volumetric flask, rinse with 0.112 M citrate buffer, and fill up to the mark.
- Proceed as described in Chapter *3.2 Solubilization of starch with dimethylsulfoxide (DMSO) and hydrochloric acid (HCl).*

3.5.4. Animal feed

- Dissolve 50 mg of animal feed in 5 mL HCl 32 % and 15 mL of DMSO.
- Heat at 60 °C (140 °F) for 180 minutes, cool rapidly to 20 – 25 °C (68 – 77 °F), and add 5 mL 8 M NaOH.
- Transfer the solution to a 50-mL volumetric flask, rinse with 0.112 M citrate buffer, and fill up to the mark.
- Proceed as described in Chapter *3.2 Solubilization of starch with dimethylsulfoxide (DMSO) and hydrochloric acid (HCl).*

3.6. Removing of oligosaccharides with ethanol

(1) Weigh 100 mg sample into a 50 mL centrifugal vial.
(2) Add 15 mL ethanol (40 % v/v), shake well and mix for 20 minutes.
(3) Centrifuge for 5 min at 4000 g.
(4) Remove the supernatant and collect it in a separate vial for determination in another Enzytec™ *Liquid* sugar assay. Dilute the supernatant 1:5 with distilled water before further use.
(5) Repeat the steps 2 – 4 at least one more time and combine the supernatants for further analysis (if needed).
(6) Add 5 mL HCl 32 %and shake well.
(7) Incubate for 60 minutes in a water bath at 60 °C (140 °F) and shake the sample well every 10 minutes.
(8) Transfer the solution into a 100 mL volumetric flask, adjust the pH to 4.8 with 8 M NaOH, and fill up to the calibration mark of the flask.
(9) Incubate the sample solution for 15 minutes at 50 – 55 °C (122 – 131 °F) before determination.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	10 – 1000 mg/L (for 100 µL sample)

Important: pre-incubate all samples at 50 – 55 °C (122 – 131 °F) for 15 minutes before measurement.		
	Reagent blank	**Samples / controls**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for **10 minutes at 20 – 37 °C** (68 – 99 °F). **Read** absorbance **A₁**, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for **10 minutes at 20 – 37 °C** (68 – 99 °F) and **read** absorbance **A₂**.		

4.1. Important notes for assay procedure

- Specified incubation times were verified and established at 37 °C (77 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of starch

The extinction difference ΔA must be calculated for each sample:

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

df:	Dilution factor
RB:	Reagent blank

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

The specified df value of **0.808 applies to a basic application of 100 µL**. An increase in sample volume is possible (max. 1000 µL; refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df)** accordingly.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The concentration of total starch is calculated using Lambert-Beer's law:

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

If the sample solution was diluted before measurement, this result has to be multiplied with the **sample pre-dilution factor F**.

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight starch [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

Results of this test Enzytec™ *Liquid* Starch E8100 additionally include the amounts of maltose, D-glucose, their oligomers and derivates as well as sucrose that might be present in the sample.

Results are 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 (free) sugars (maltose, D-glucose, their oligomers and derivatives as well as sucrose) must be determined using the test Enzytec™ *Liquid* Maltose/Sucrose/D-Glucose (E8170).

This result is expressed as total maltose (342.3 g/mol) and subtracted from total starch for differentiation:

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

Important: The starch and maltose assays require different sample preparations and the extracts cannot be interchanged. For later calculations, it is therefore important to ensure that the extracted samples are at the same dilution. As an alternative, calculations can be made using the weight per 100 g.

5.2. Calculation of starch content in solid samples

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

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

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. The recovery of starch control solutions and for extracted starch samples should be within 100 ± 10 %.

We recommend a commercially available starch material with known purity and water content. For control or spike solutions, the following materials can be used:

- Corn starch, Carl Roth (Art. No. 4701.1)
- Potato starch, Sigma Aldrich (Art. No. 33615-250G)
- Wheat starch, Merck (Art. No. 1.11685.0250)
- Rice starch, Sigma Aldrich (Art. No. S7260-500G)

To prepare a 1 g/L control solution, follow the instructions for sample preparation in section *3.2 Solubilization of starch with dimethylsulfoxide (DMSO) and hydrochloric acid (HCl)*.

Important: The water content of the sample must be taken into account.

Example:

According to the certificate, corn starch (Carl Roth) has a moisture content of12,5 % (100 % − 12.5 % = 87.5 % → factor: 0.875).

If a starch solution is prepared with a concentration of 0.72 g/L of cornstarch, the actual starch concentration is 0.63 g/L (0.72 g/L × 0.875 = 0.63 g/L).

6. Performance data

6.1. Specificity & side activities

Amyloglucosidase hydrolyzes α-1,4-glycosidic 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 %. The included hexokinase is specific for D-glucose. In addition, the side activity for sucrose was determined to be 47 %, and for lactose and raffinose, 7 % respectively, in the starch assay.

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 *5.1.2. Calculation of the real starch concentration*.

6.2. Interferences

At concentrations of 2 g/L or less, no interference was observed for sulfur dioxide (SO_2) and ascorbic acid.

Citric acid does not interfere at concentrations of 50 g/L or less.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 1000 mg/L total starch (sample volume of 100 µL) with a recommended measuring range of 10 – 1000 mg/L.

The limit of detection (LoD) was determined according to the DIN 32645:2008-11 method in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 3.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 10.0 mg/L for a sample volume of 100 µL.

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* Starch Validation Report
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Starch Excel template for results
- Enzytec™ *Liquid* Starch Technical information
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

Upon request, we offer the following services, among others:

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

10. Disclaimer

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