

Product Information

Starch Assay Kit

(Amylase/Amyloglucosidase Method)

Catalog Number **STA20**

Storage Temperature 2–8 °C

(Do Not Freeze)

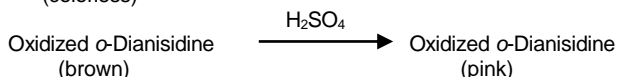
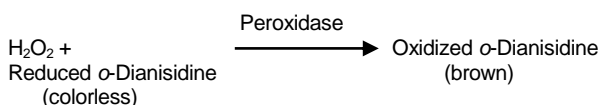
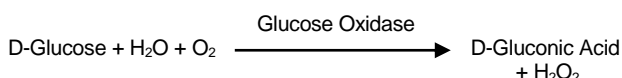
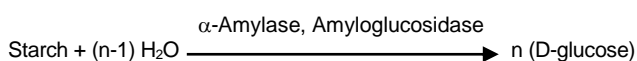
TECHNICAL BULLETIN

Product Description

Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industries. Enzymatic methods are specific, reproducible, sensitive, rapid, and therefore, ideal for analytical purposes. Because of the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.¹⁻⁹

This kit is for the quantitative, enzymatic determination of starch in food and other materials. Several publications have noted use of this kit on such samples as plant leaves,¹⁰ tomato,¹¹ and algae.¹²

Principle



- The hydrolysis of starch to glucose is catalyzed by α -amylase and amyloglucosidase.
- Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase.
- Hydrogen peroxide reacts with α -dianisidine in the presence of peroxidase to form a colored product.
- Oxidized α -dianisidine reacts with sulfuric acid to form a more stable colored product.
- The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration.

Reagents

1. α -Amylase for Starch Assay Kit (Catalog Number A4582): This heat stable α -amylase is supplied as a solution in 25% propylene glycol and is ready-to-use.
2. Starch Assay Reagent (Catalog Number S9144): Reconstitute the contents of the vial with 20 mL of water. After addition of water, stopper the vial and mix several times by inversion. **Do Not Shake.**

Each vial, when reconstituted with 20 mL of water, contains 50 units/mL of amyloglucosidase from *Aspergillus niger* and buffer salts.

The reconstituted reagent is stable in the absence of visible microbial growth for 7 days at 18–26 °C and for 4 weeks at 2–8 °C. The dry reagent should be discarded under these circumstances:

- if the vial exhibits caking because of possible moisture penetration,
- if the vial contents do not dissolve completely upon reconstitution,
- or if the solution appears turbid.

3. Glucose Oxidase/Peroxidase Reagent (Catalog Number G3660): Each capsule contains 500 units of glucose oxidase from *Aspergillus niger*, 100 purpurgalin units of horseradish peroxidase, and buffer salts. Store the unopened reagent at 2–8 °C.

Empty the contents of the capsule into an amber bottle. Dissolve the contents in 39.2 mL of water. The solution is stable up to one month at 2–8 °C and for at least 6 months frozen at –20 °C. Discard if turbidity develops.

4. *o*-Dianisidine Reagent (Catalog Number D2679): The preweighed vial contains 5 mg of *o*-dianisidine dihydrochloride. Store the reagent at 2–8 °C and minimize exposure to light.

Reconstitute the contents of the *o*-dianisidine vial with 1.0 mL of water. Invert the vial several times to dissolve the contents. The solution is stable for 3 months at 2–8 °C. Store the solution to minimize exposure to light.

5. Glucose Assay Reagent: Add 0.8 mL of the reconstituted *o*-Dianisidine Reagent to the amber bottle which contains the 39.2 mL of the reconstituted Glucose Oxidase/Peroxidase Reagent. Invert the bottle several times to mix. Store the reagent at 2–8 °C, and minimize exposure to light. The solution is stable up to 1 month at 2–8 °C. Discard if turbidity develops or color forms.
6. Glucose Standard Solution (Catalog Number G3285): D-Glucose, 1.0 mg/ml in 0.1% benzoic acid. This standard is **traceable to an NIST standard** and is supplied ready-to-use. It is stable at 2–8 °C for at least six months. Discard if turbidity develops.
7. Wheat Starch, Standard for Starch Assay Kit (Catalog Number S1520): This standard is used as a control to ensure assay reliability. The dry reagent is stable for at least 2 years when stored desiccated at room temperature. Moisture content will vary depending on storage conditions.
8. Corn Starch, Standard for Starch Assay Kit (Catalog Number S5296): This standard is used as a control to ensure assay reliability. The dry reagent is stable for at least 2 years when stored desiccated at room temperature. Moisture content will vary depending on storage conditions.

Reagents and Equipment Required but Not Provided

- 6 M Sulfuric Acid Solution – Prepared by a 3-fold dilution in water of concentrated (18 M) sulfuric acid, ACS Reagent (e.g. Catalog Number 258105)
- 80% Ethanol Solution – Prepared by dilution of 95% ethanol with water.
- Dimethyl Sulfoxide (DMSO), ACS Reagent (e.g. Catalog Number 154935)

Apparatus

- Spectrophotometer suitable for measuring absorbance at 540 nm
- Cuvettes
- Test Tubes, 16 mm × 120 mm
- Pipettes capable of accurately dispensing 10 µL to 10 mL
- Boiling water bath
- Water bath capable of maintaining temperatures at 60 ± 1 °C and 37 ± 1 °C
- Analytical centrifuge
- Analytical balance
- Vortex mixer

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

Grind the sample to < 0.5 mm (No. 40 mesh). Weigh 50–100 mg samples to 0.1 mg accuracy. Transfer the samples to appropriately marked test tubes.

For wheat and corn starch controls, and samples with high starch content, reduce the sample size to 1–10 mg.

Samples that contain glucose or maltodextrins must be extracted with ethanol to remove these substances.

1. Add 5.0 mL of the 80% Ethanol Solution to the sample.
2. Incubate at 80–85 °C for 5 minutes.
3. Mix the contents of the tube and add another 5.0 mL of the 80% Ethanol Solution.
4. Centrifuge tube for 10 minutes at $1,000 \times g$. Discard the supernatant.
5. Resuspend the pellet in 10 mL of the 80% Ethanol Solution and mix. Centrifuge for 10 minutes at $1,000 \times g$. Carefully pour off the supernatant and discard.
6. Proceed with starch digestion in the next section.

For samples that contain resistant starch:

1. Add 2 mL of DMSO to each sample.
2. Mix and incubate for 5 minutes in a boiling water bath.
3. Proceed with starch digestion in the next section.

Procedure

Starch Digestion

1. Add 0.2 mL of the 80% Ethanol Solution to each sample and to an empty tube labeled "Starch Digestion Blank" and mix.
2. Pipette 3.0 mL of water and 0.02 mL of the α -Amylase (Reagent 1) into each sample and blank tube.
3. Mix and incubate for 5 minutes in a boiling water bath.
4. Remove the tubes from the water bath and cool to room temperature.
5. Bring the volume in each tube up to 10 mL with water and mix.
6. To 1.0 mL of each test and blank solution from step 5, add 1.0 mL of the Starch Assay Reagent (Reagent 2).
7. Mix and incubate for 15 minutes in a 60 °C shaking water bath.
8. Remove the tubes from the water bath and cool to room temperature.
9. Dilute 1.0 mL of each sample and blank to 10 mL with water.
10. Proceed with glucose determination in the next section.

Glucose Assay

Avoid prolonged exposure of the Glucose Assay Reagent (Reagent 5) to bright light.

1. Pipette the following solutions into the appropriately marked test tubes:

Reagent	Standard Blank	Standard	Reagent Blank	Test
Water (mL)	1.0	0.950	---	---
Glucose Standard - Reagent 6 (mL)	---	0.05	---	---
Blank from Starch Digestion (mL)	---	---	1.0	---
Sample from Starch Digestion (mL)	---	---	---	1.0

2. At time zero, start the reaction by adding 2.0 mL of the Glucose Assay Reagent (Reagent 5) to the first tube and mix. Allow 30–60 second intervals between addition of Glucose Assay Reagent (Reagent 5) to each subsequent tube.
3. Incubate each tube exactly 30 minutes at 37 °C. Stop each reaction at 30–60 second intervals by adding 2.0 mL of the 6 M Sulfuric Acid Solution into each tube. Carefully mix each tube thoroughly.
4. Measure the absorbance of each tube at 540 nm.

Calculations

$$\Delta A_{\text{STANDARD}} = A_{\text{STANDARD}} - A_{\text{STANDARD BLANK}}$$

$$\Delta A_{\text{TEST}} = A_{\text{TEST}} - A_{\text{REAGENT BLANK}}$$

%Starch

$$= \frac{(\Delta A_{\text{TEST}})(F)(V)(SF)(SDF)(VGA)(MWF)(100)}{(\text{Conversion Factor for } \mu\text{g to mg})(\text{sample Weight in mg})}$$

$$= \frac{(\Delta A_{\text{TEST}})(50/\Delta A_{\text{STD}})(10)(2)(10)(1.0)(0.9)(100)}{(1000)(\text{Sample Weight in mg})}$$

$$= \frac{(\Delta A_{\text{TEST}})(900)}{(\Delta A_{\text{STD}})(\text{Sample Weight in mg})}$$

F = μg glucose in standard \div $\Delta A_{\text{STANDARD}}$ at 540 nm = $50/\Delta A_{540}$

V = Initial Sample Volume (from sample preparation)

SF = Total Assay Volume from Starch Assay/Sample Volume from Starch Assay

SDF = Dilution Factor from end of Starch Assay

VGA = Initial Sample Volume from Glucose Assay

MWF = Molecular Weight of Starch monomer/Molecular Weight of Glucose = $162/180 = 0.9$

References

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CP,CMH,GCY,MAM 10/19-1