STARCH ASSAY KIT
(Amylase/Amyloglucosidase Method)
Product Number **STA-20**
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. Due to the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.

This kit is for the quantitative, enzymatic determination of starch in food and other materials.

Principle

$$\text{Starch} + (n\text{-}1)\ H_2O \xrightarrow{\alpha\text{-Amylase, Amyloglucosidase}} n\ \text{glucose}$$

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D-Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Reduced o-Dianisidine (colorless)} \xrightarrow{\text{Peroxidase}} \text{Oxidized o-Dianisidine (brown)}$$

$$\text{Oxidized o-Dianisidine (brown)} \xrightarrow{H_2SO_4} \text{Oxidized o-Dianisidine (pink)}$$

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 o-dianisidine in the presence of peroxidase to form a colored product. Oxidized o-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

Refer to Material Safety Data Sheets for updated risk, hazard or safety information.

1. **α-Amylase for Starch Assay Kit** (Sigma Product No. A 4582)
   Solution in 25% propylene glycol.

2. **Starch Assay Reagent** (Sigma Product No. S 9144)
   Reconstitute vial with 20 ml of deionized water. After addition of deionized water, stopper vial and mix several times by inversion. DO NOT SHAKE.

   Each vial, when reconstituted with 20 ml of deionized 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. Dry reagent should be discarded if the vial exhibits caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution or if the solution appears turbid.

3. **Glucose Oxidase/Peroxidase Reagent** (Sigma Product No. G 3660)
   Store the unopened reagent at 2–8°C. Each capsule contains 500 units of glucose oxidase from *Aspergillus niger,* 100 Purpurgalin units of horseradish peroxidase, and buffer salts.

   In an amber bottle, dissolve the contents of the capsule in 39.2 ml of deionized 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** (Sigma Product No. D 2679)
   Minimize exposure to light.
   Store the unopened reagent at 2–8°C. The pre-weighed vial contains 5 mg of o-dianisidine dihydrochloride.

   Reconstitute the vial of o-dianisidine with 1.0 ml of deionized water. Invert the vial several times to dissolve. The solution is stable for 3 months at 2–8°C.

5. **Glucose Assay Reagent**
Add 0.8 ml of the o**-Dianisidine Reagent** to the amber bottle containing the 39.2 ml of **Glucose Oxidase/Peroxidase Reagent.** Invert bottle several times to mix. 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** (Sigma Product No. G 3285)
D-Glucose, 1.0 mg/ml in 0.1% benzoic acid. Store reagent at 2–8°C. Supplied ready to use. The solution is stable at 2–8°C for at least six months.

7. **Wheat Starch, Standard for Starch Assay Kit** (Sigma Product No. S 1520)
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** (Sigma Product No. S 5296)
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 Not Included in Kit

1. **Sulfuric Acid, ACS reagent** (Sigma Product No. S 1526)
Reagent is 36N sulfuric acid. Prepare a 12N solution in deionized water.

2. Ethanol, 80% (v/v)
Prepare solution in deionized water.

3. Dimethyl Sulfoxide (DMSO) (Sigma Product No. D 8779)

Apparatus
1. Spectrophotometer suitable for measuring absorbance at 540 nm.
2. Cuvets
3. Test Tubes, 16 mm x 120 mm
4. Pipets capable of accurately dispensing 10 µl to 10 ml.
5. Boiling water bath.
6. Water bath capable of maintaining temperatures at 60 ± 1°C and 37 ± 1°C.
7. Analytical centrifuge.
8. Analytical balance.
9. Vortex mixer.

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

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

1. Add 5.0 ml of 80% ethanol to the sample.

2. Incubate at 80–85°C for 5 minutes.

3. Mix tube contents and add another 5.0 ml of 80% ethanol.

4. Centrifuge tube for 10 minutes at 1000 g. Discard supernatant.

5. Resuspend pellet in 10 ml of 80% ethanol and mix. Centrifuge for 10 minutes at 1000 g. Carefully pour off supernatant and discard.

6. Proceed with starch determination in next section.

Samples that contain resistant starch:
1. Add 2 ml DMSO to each sample.

2. Mix tubes and incubate for 5 minutes in a boiling water bath.

3. Proceed with starch determination in next section.

Determination

Starch Digestion

1. Add 0.2 ml of 80% ethanol to each sample and to a blank tube and mix.

2. Pipet 3.0 ml of deionized water and 0.02 ml of α-Amylase (Reagent 1) into each sample and blank tube.

3. Mix the tubes and incubate for 5 minutes in a boiling water bath.

4. Remove the tubes from water bath and cool to room temperature.

5. Bring the volume in each tube up to 10 ml with deionized water and mix.

6. To 1.0 ml of each test and blank solution from step #5, add 1.0 ml of Starch Assay Reagent (Reagent 2).

7. Mix the tubes 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 deionized water.

10. Proceed with glucose determination in next section.

Glucose Assay

Avoid prolonged exposure of the **Glucose Assay Reagent** to bright light.

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

Reagent	Standard Blank	Standard	Reagent Blank	Test
Deionized Water (ml)	1.0	0.950	—	—
Glucose Standard Solution, Reagent 6 (ml)	—	0.05	—	—
Blank from Starch Digestion (ml)	—	—	1.0	—
Sample from Starch Digestion (ml)	—	—	—	1.0

2. At zero time, start the reaction by adding 2.0 ml of **Glucose Assay Reagent** (Reagent 5) to the first tube and mixing. Allow a 30 to 60 second interval between additions of **Glucose Assay Reagent** (Reagent 5) to each subsequent tube.

3. Incubate each tube exactly 30 minutes at 37 C. Stop reaction at 30–60 second intervals by adding 2.0 ml of **12 N H_2SO_4** into each tube. Carefully mix each tube thoroughly.

4. Measure the absorbance of each tube at 540 nm.

Calculations

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

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

$$= (\Delta A_{TEST})\,(9000) \,/\, (\Delta A_{STD})\,(\text{Sample Weight in mg})$$

$\Delta A_{STANDARD} = A_{STANDARD} - A_{STANDARD\ BLANK}$
$\Delta A_{TEST} = A_{TEST} - A_{REAGENT\ BLANK}$
F = µg glucose in standard ÷ $\Delta A_{STANDARD}$ 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
VGA = Initial Sample Volume from Glucose Assay
SDF = Dilution Factor from end of Starch Assay
MWF = Molecular Weight of Starch/Molecular Weight of Glucose = 162/180 = 0.9

References

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