

Product Information

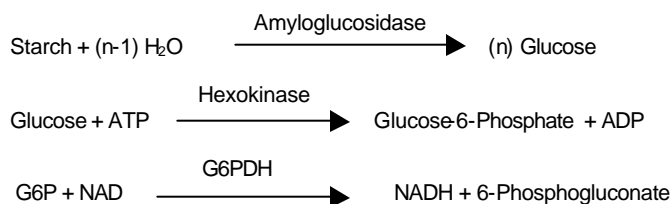
STARCH ASSAY KIT

Product Code **SA-20**

TECHNICAL BULLETIN

Product Description

Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industry. 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 native starch in food and other materials.



The hydrolysis of starch to glucose is catalyzed by amyloglucosidase. Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Glucose-6-phosphate (G6P) is then oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in absorbance at 340 nm is directly proportional to the glucose concentration.

Reagents

1. Starch Assay Reagent (Product Code S 9144)
Reconstitute vial with 20 ml of deionized water. After addition of deionized water, stopper vial and immediately mix several times by inversion. DO NOT SHAKE. Each vial when reconstituted with 20 ml of deionized water contains 50 U/ml of amyloglucosidase (*Aspergillus niger*) and buffer salts. The reconstituted reagent is stable for 7 days at 18-26 °C and for 4 weeks at 2-8 °C.

2. Glucose (HK) Assay Reagent (Product Code G 3293)
Reconstitute the vial contents with 20 ml of water. After addition of water, stopper the vial and immediately mix several times by inversion. DO NOT SHAKE.

Each vial when reconstituted with 20 ml of water contains 1.5 mM NAD, 1.0 mM ATP, 1.0 unit/ml of hexokinase, and 1.0 unit/ml of glucose-6-phosphate dehydrogenase with sodium benzoate and potassium sorbate as preservatives.

The dry reagent is stored at 2–8 °C. The reagent should be discarded if the vial contents exhibit caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution, or if the reconstituted solution appears turbid.

The reconstituted reagent is stable, in the absence of visible microbial growth for 7 days at 18–26 °C and for at least 4 weeks at 2–8 °C. The reagent is not suitable for use if the absorbance of the freshly reconstituted solution measured at 340 nm versus water as the reference is greater than 0.350.

2. Starch Assay Standard (Product Code S 5296)
Used as a control to ensure assay reliability. Dry reagent is stable for at least 2 years stored desiccated at room temperature. Moisture content will vary depending on storage conditions.

Equipment Required but Not Provided

1. Spectrophotometer suitable for measuring absorbance at 340 nm.
2. Cuvettes
3. Test Tubes, 13 mm X 100 mm
4. Pipettes capable of accurately dispensing 10 µl to 2 ml.
5. Water bath capable of maintaining temperature at 60±1 °C

Precautions

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

ProcedureSample Preparation

Liquids: Use without additional preparation.

Solids: Grind sample to <0.5 mm (No. 40 mesh).
Weigh a 0.1 - 1 gram sample to 0.1 mg accuracy.

Use one of the methods detailed below to solubilize the sample:

Method #1 DMSO/HCl

1. Transfer sample into a flask (100-250 ml) and add 20 ml of DMSO and 5 ml of 8 M HCl.
2. Incubate covered flask for 30 minutes at 60 °C in a shaking water bath.
3. Add 50 ml of deionized water to flask and then adjust pH to pH 4-5 with 5 N NaOH.
4. Cool solution to room temperature and dilute to 100 ml with deionized water.

Method #2 Autoclave

1. Transfer sample into a flask (100-150 ml).
2. With stirring, add 25 ml of deionized water.
3. Check pH and adjust, if necessary, to pH 5-7.
4. Boil with gentle stirring for 3 minutes.
5. Autoclave for 1 hour at 135 °C.
6. Remove solution from autoclave after the cycle is complete and temperature has fallen to about 60 °C.
7. Add deionized water to a total volume of 100 ml.

Starch Assay

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

Tube	Starch Assay Reagent (ml)	Sample (ml)	Deionized Water (ml)
Starch Assay Reagent Blank	1.0	----	1.0
Sample Blank	----	1.0	1.0
Glucose Assay Reagent Blank	----	----	2.0
Test	1.0	1.0	----

2. Mix tubes and incubate for 15 minutes at 60 °C in a shaking water bath.
3. Remove tubes from water bath and cool to room temperature.

Glucose Assay

Sample volume for this assay will vary depending on the starch content and weight of the original sample. Pipette a volume of solution corresponding to a glucose content of approximately 0.5-50 µg. Repeat the assay and vary the sample volume if necessary to give a ΔA_{340} between 0.03 and 1.6.

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

Tube	Glucose Assay Reagent (ml)	Sample Volume in µl (Solutions from Starch Assay)
Starch Assay Reagent Blank	1.0	Same as for Test
Sample Blank	1.0	Same as for Test
Glucose Assay Reagent Blank	1.0	Same as for Test
Test	1.0	10 - 200

2. Mix tubes and incubate for 15 minutes at room temperature (18-35 °C).
3. Measure the absorbance at 340 nm.

Calculations

Total Blank

The total blank must take into account the contribution to the absorbance of the sample, the glucose assay reagent and the starch assay reagent. The absorbance of the glucose assay reagent is subtracted from the sample blank so that the absorbance of the glucose assay reagent is only counted once in the total absorbance since it is in both the sample blank and the starch assay reagent blank.

$$A_{\text{Total Blank}} = (A_{\text{Sample Blank}} - A_{\text{Glucose Assay Reagent Blank}}) + A_{\text{Starch Assay Reagent Blank}}$$

Starch concentration

Starch concentration in mg/ml = SC

$$SC = \frac{(\Delta A) (TVSA/SVSA) (TVGA/SVGA) (\text{Starch MW}) (F)}{(\epsilon) (d) (1,000)}$$

$$\Delta A = A_{\text{Test}} - A_{\text{Total Blank}}$$

TVSA = Total Assay Volume from Starch Assay in ml

SVSA = Sample Volume from Starch Assay in ml

TVGA = Total Assay Volume from Glucose Assay in ml

SVGA = Sample Volume from Glucose Assay in ml

Starch MW = 162.1 g/mole or equivalently

$$162.1 \mu\text{g}/\mu\text{moles}$$

F = Dilution Factor from Sample Preparation

ϵ = Millimolar Extinction Coefficient for NADH at 340 nm
Millimolar⁻¹ cm⁻¹ or equivalently (ml/ μ moles)(1/cm)

d = Light path (cm) = 1 cm

1,000 = Conversion Factor for μ g to mg

$$SC \text{ (mg/ml)} = \frac{(\Delta A) (2) (TVGA/SVGA) (162.1) (F)}{(6.22) (1) (1,000)}$$

$$SC \text{ (mg/ml)} = (\Delta A) (TVGA/SVGA) (F) (0.052)$$

References

1. Beutler, H.O., Methods of Enzymatic Analysis, Bergmeyer, H.U., ed., New York, Academic Press, 3rd Edition, **6**, 2-10 (1984).
2. MacRae, J.C., J. Sci. Fd. Agric., **25**, 1465 -1469 (1974).
3. Methods of Analysis of the AOAC, 16th Edition (1995) section 32.2.05.
4. Southgate, D.A.T., Determination of Food Carbohydrates, Applied Science Publishers, London (1976).
5. Thivend, P., et al., Methods in Carbohydrate Chemistry, **6**, 100-105 (1972).

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