3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-521-8956 • (314) 771-5765
Fax 800-325-5052 • (314) 771-5757
sigma-aldrich.com

ProductInformation

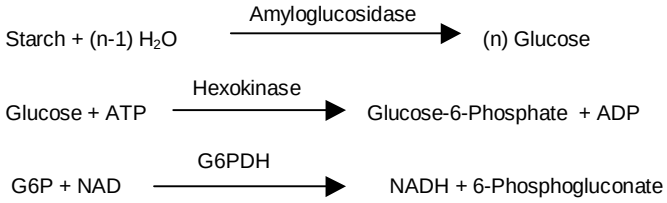
STARCH ASSAY KIT

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 2020)
   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 1.5 mM NAD, 1.0 mM ATP, 1.0 U/ml hexokinase, and 1.0 U/ml of glucose-6-phosphate dehydrogenase with 0.05% sodium azide as a preservative. The dry kit reagent is stored at 2-8 °C. The reconstituted reagent is stable for 7 days at 18-26 °C and for 4 weeks at 2-8 °C. The reagent is not suitable for use if the absorbance of a freshly reconstituted solution measured at 340 nm vs water as reference is greater than 0.350.
3. 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. Cuvets
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

Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

Sample 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.

Sample Preparation (continued): Use one of the methods detailed below to solubilize 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

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

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

$$\text{mg of Starch} = \frac{(\Delta A)\,(TVSA/SVSA)\,(TVGA/SVGA)\,(\text{Starch MW})\,(F)}{(\varepsilon)\,(d)\,(\text{Conversion Factor for } \mu g \text{ to mg})}$$

$$= \frac{(\Delta A)\,(2)\,(TVGA/SVGA)\,(162.1)\,(F)}{(6.22)\,(1)\,(1000)}$$

$$= (\Delta A)\,(TVGA/SVGA)\,(F)\,(0.052)$$

TVSA = Total Assay Volume from Starch Assay
SVSA = Sample Volume from Starch Assay
TVGA = Total Assay Volume from Glucose Assay
SVGA = Sample Volume from Glucose Assay
Starch MW = 162.1 d = Light path (cm)
F = Dilution Factor from Sample Preparation
ε = Millimolar Extinction Coefficient for NADH at 340 nm

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 Carbohyrates, Applied Science Publishers, London (1976).
5. Thivend, P., et al., Methods in Carbohydrate Chemistry, **6**, 100-105 (1972).

CMH/MAM 8/02