Technical Bulletin

Starch Assay Kit

Catalogue number MAK522

Product Description

Starch, chemical formula $(C_6H_{10}O_5)_n$, is a polysaccharide carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. All plant seeds and tubers contain starch present in the form of amylose and amylopectin. Starch is the most consumed polysaccharide in the human diet. Some starches are digested very quickly and cause a rapid and large rise in blood sugar. Others are digested more slowly, and some starch, called resistant starch, is not digested in the small intestine at all, and thus causes little or no blood sugar rise. Simple, direct and automation-ready procedures for measuring starch concentrations find wide applications in research and drug discovery.

The Starch Assay Kit uses a single Working Reagent that combines the enzymatic breakdown of starch and the detection of glucose in one step. The color intensity of the reaction product at 570 nm or fluorescence intensity at λex =530 nm / λem = 585 nm is directly proportional to the starch concentration in the sample. This simple convenient assay is carried out at room temperature and takes only 30 min.

The linear detection range of the kit is 2 to 200 µg/mL starch for colorimetric assays and 0.2 to 20 µg/mL for fluorometric assays.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 12 mL
  Catalogue Number MAK522A
- Dye Reagent 120 µL
  Catalogue Number MAK522B
- Enzyme A 1 vial
  Catalogue Number MAK522C
- Enzyme B 120 µL
  Catalogue Number MAK522D
- Standard (50 mg/mL) 50 µL
  Catalogue Number MAK522E

Equipment Required but Not Provided

- Pipetting devices and accessories (For example - multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- Centrifuge tubes Dounce tissue grinder set (Catalog Number D9063 or equivalent)

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.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Enzyme A: Reconstitute Enzyme A by adding 120 µL Assay Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. During the experiment, keep thawed enzymes in a refrigerator or on ice. Store reconstituted Enzyme A at -20 °C and use within 1 month.

Note: 1. This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the working reagent is recommended.

2. SH-group containing reagents (For example - DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.

Procedure

All Samples and Standards should be run in duplicate.

Reagent Preparation

Soluble Starch:

1. Use 5-10 mg of the Sample and grind followed by washing off any free glucose and small oligosaccharides with 1 mL 90% ethanol.
2. Warm to 60 °C for 5 min with occasional vortexing. Centrifuge at 10,000g for 2 min.
3. Decant the supernatant.
4. Repeat the wash twice. Remove ethanol.

Soluble starch in the pellet form:

1. Extract with 1 mL H_2O incubated in a boiling water bath for 5 min.
2. Spin 10,000 x g for 2 min.
3. The supernatant is soluble starch and resistant starch remains as an insoluble pellet.

Resistant Starch:

1. After extracting soluble starch, extract the water insoluble pellet with 0.2 mL DMSO and heat in boiling water bath for 5 min.
2. Dilute sample 1:100 in H_2O prior to assay.

Colorimetric Procedure

Transfer 10 µL of Sample into separate wells of a clear flat-bottom microplate.

Standard curve preparation:

Prepare a 200 µg/mL Standard by diluting 5 µL of 50 mg/mL Standard with 1.245 mL purified water. Further, dilute Standards as mentioned in Table 1.

Table 1. Dilution of Standard

Well No.	200 µg/mL STD (µL)	Purified Water (µL)	Starch Concentration (µg/mL)
1	200	0	200
2	150	50	150
3	100	100	100
4	50	150	50
5	0	200	0

Transfer 10 µL of the Standards and Samples into separate wells of a clear flat-bottom microplate.

Note: If the Sample contains glucose, transfer an additional 10 µL Sample to another well for the Sample Blank.

Working Reagent Preparation

For each Standard and Sample well, prepare enough Working Reagent according to Table 2.

Table 2.

Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	90 µL	85 µL
Enzyme A	1 µL	-
Enzyme B	1 µL	1 µL
Dye Reagent	1 µL	1 µL

Transfer 90 µL Working Reagent into each reaction well. Add 90 µL Blank Working Reagent to the Sample Blank Wells. Tap plate to mix.

Measurement

1. Incubate for 30 mins at Room Temperature.
2. Read the optical density at 570nm (550-585 nm)

Fluorometric Procedure

1. Prepare a 200 µg/mL Standard by diluting 5 µL of 50 mg/mL Standard with 1.245 mL purified water. Further, dilute Standards as mentioned in Table 1.

Table 1. Dilution of Standard

Well No.	200 µg/mL STD (µL)	Purified Water (µL)	Starch Concentration (µg/mL)
1	20	180	20
2	15	185	15
3	10	190	10
4	5	195	5
5	0	200	0

2. Repeat the same procedure as performed in the Colorimetric procedure except use a use a black flat-bottom microplate.
3. Incubate for 30 min at room temperature and read fluorescence at λ_{ex} = 530 nm / λ_{em} = 585 nm.

Results

1. Subtract the blank value from all the standard values.
2. Plot the ΔF or ΔOD against the standard concentrations.
3. Determine the slope and calculate Starch concentration of samples using the below given equation:

$$\text{Starch (µg/mL)} = \frac{R_{Sample} - R_{Blank}}{\text{Slope}}$$

Where:

R_{Sample} = Optical Density (OD) or Fluorescence Intensity (F) of the Sample

R_{Blank} = Optical Density (OD) or Florescence Intensity (F) of the Blank

Figure 1.
Typical standard curve from Colorimetric Assay.

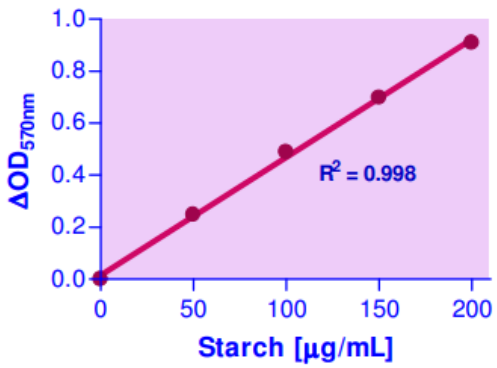
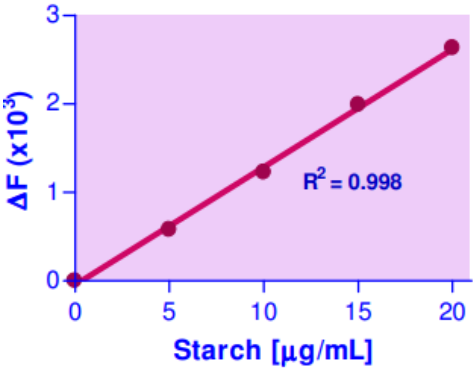


Figure 2.

Typical standard curve from Fluorometric Assay.



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