Product Manual

Starch Assay Kit (Fluorometric)

Catalog Number

MET-5026 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Starch is a polymeric carbohydrate made of a long chain of glucose units joined together by covalent glycosidic bonds. This polymer is created by most green plants as a form of reserve energy. Plants make starch by using the enzyme glucose-1-phosphate adenylyltransferase to convert glucose 1-phosphate to ADP-glucose (this initial step requires ATP as a source of energy). ADP-glucose is then added to the elongating chain of glucose residues by the enzyme starch synthase. This step releases ADP and creates amylose. Finally, a starch branching enzyme creates 1,6-alpha glycosidic bonds between these chains, creating the branched amylopectin molecule.

Starch is the most common carbohydrate in the diet of humans and can be found in many common foods such as potatoes, corn, wheat, and rice. Foods such as potato have a higher glycemic index rating due to high amounts of digestible starch, thus increasing the risk of type-2 diabetes and cardiovascular disease in consumers. Food starches are often used as thickeners and stabilizers in foods such as soups, sauces, puddings, custards, gravies, pie fillings, and salad dressings, as well as to make various types of noodles. Starch is often converted into sugar which can be used in processed foods. Starch is used in the paper industry as an adhesive, and often is used to stiffen some parts of clothing just prior to ironing.

Cell Biolabs' Starch Assay Kit is a simple fluorometric assay that measures the amount of total starch present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, starch standards, and unknown samples. Sample starch concentrations are determined by comparison with a known starch standard. The kit has a detection sensitivity limit of 625 ng/mL starch.

Note: Each sample replicate requires 2 assays, one treated with amyloglucosidase (+AG) and one without (-AG). Starch is calculated from the difference in RFU readings from the 2 wells.

Assay Principle

Cell Biolabs' Starch Assay Kit measures total starch within biological samples. Starch is broken down into glucose monomers by amyloglucosidase first, glucose is then oxidized by glucose oxidase into D-gluconic acid and hydrogen peroxide. The resulting hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of Starch standard within the 96-well microtiter plate format. Samples and standards are incubated for 45 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).

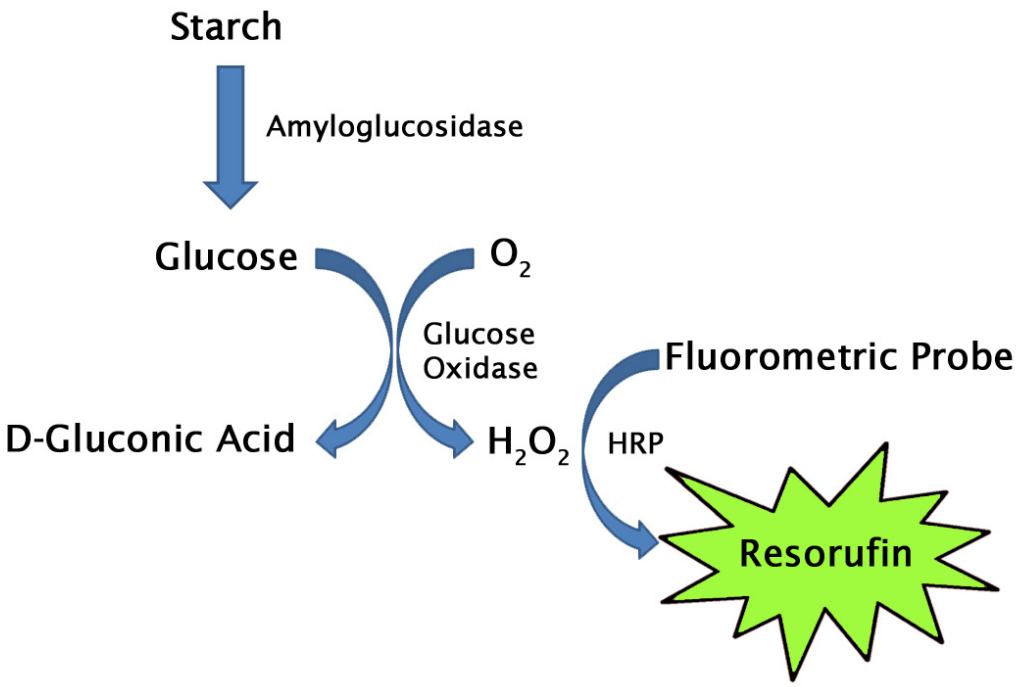

Figure 1. Starch Assay Principle.

Related Products

1. MET-5022: Glycogen Assay Kit (Colorimetric)
2. MET-5023: Glycogen Assay Kit (Fluorometric)
3. MET-5001: Lactose Assay Kit
4. MET-5012: Lactate Assay Kit (Colorimetric)
5. STA-398: Free Glycerol Assay Kit (Colorimetric)
6. STA-680: Glucose Assay Kit (Colorimetric)
7. STA-682: Total Carbohydrate Assay Kit
8. STA-670: Homocysteine ELISA Kit
9. STA-674: Glutamate Assay Kit
10. STA-675: Hydroxyproline Assay Kit

Kit Components

1. Starch Standard (Part No. 50251C): One 50 µL tube at 2 mg/mL.
2. 10X Assay Buffer (Part No. 268002): One 25 mL bottle of 500 mM sodium phosphate pH 7.4.
3. Fluorometric Probe (Part No. 50231C): One 50 µL tube in DMSO.
4. HRP (Part No. 234402-T): One 10 µL tube of a 100 U/mL solution in glycerol.
5. Amyloglucosidase (Part No. 50223C): One 1 mL tube at 15 U/mL.

Note: One unit is defined as the amount of enzyme that will release 1.0 micromole of glucose per minute at pH 4.8 at 60°C.

6. Glucose Oxidase (Part No. 50015C): One 100 µL tube at 200 U/mL.

   Note: One unit is defined as the amount of enzyme that will oxidize 1.0 micromole of beta-D-glucose to D-gluconic acid and hydrogen peroxide per minute at pH 5.1 at 35°C.

Materials Not Supplied

1. Distilled or deionized water
2. Phosphate Buffered Saline (PBS)
3. 2 N NaOH
4. 2 N HCl
5. 1 M Tris Base pH 6.0
6. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
7. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
8. Standard 96 well fluorescence black microtiter plate and/or black cell culture microplate
9. Multichannel micropipette reservoir
10. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range

Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Store all other components at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Note: After thawing Amyloglucosidase for the first time, make smaller aliquots and store at -20°C.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, and Glucose Oxidase 1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, and 20 µL of Glucose Oxidase to 968 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Food samples (including rice, wheat, corn and potato): Weigh out 20 mg of sample (for example one grain of rice). Add sample to a 2 mL screw cap tube. Add 400 µL of 2 N NaOH and grind up

the solid (within the solution) into smaller fragments using a spatula. Close the tube tightly and incubate at 95°C for 30 minutes in a hot water bath. Cool for 2-3 minutes at 4°C. Spin at 12000 x g for 2 minutes and remove supernatant. Transfer the supernatant to a new tube. Repeat the extraction procedure 2-3 more times using the pellet until no more pellet is observed. Pool the supernatant together into one tube and calculate the volume. Add an equal volume of 2 N HCl followed by an equal volume 1 M Tris Base pH 6.0 and mix well. (For example, to 800 µL of pooled supernatant, add 800 µL of 2 N HCl followed by 800 µL of 1 M Tris Base pH 6.0). Store the neutralized extraction samples at -20°C until ready to test in the assay.

Notes:

- *Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).*
- *All samples should be assayed immediately or stored at -20°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Starch standards before use by diluting the 2 mg/mL Starch Standard in PBS according to Table 1 below.

Standard Tubes	2 mg/mL Starch Standard(µL)	1X PBS (µL)	Starch (µg/mL)
1	10	490	40
2	250 of Tube #1	250	20
3	250 of Tube #2	250	10
4	250 of Tube #3	250	5
5	250 of Tube #4	250	2.5
6	250 of Tube #5	250	1.25
7	250 of Tube #6	250	0.625
8	0	250	0

Table 1. Preparation of Starch Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with Amyloglucosidase and one without the enzyme to measure endogenous glucose background (PBS will be added in place of Amyloglucosidase).

2. Add 50 µL of each Starch standard or unknown sample into wells of a fluorescence black 96-well microtiter plate.
3. Add 10 µL of Amyloglucosidase to the standards and to one half of the paired sample wells and mix the well contents thoroughly.
4. Add 10 µL of PBS to the other half of the paired sample wells and mix thoroughly.
5. Incubate for 30 minutes at 37°C.
6. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 45 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
7. Read the plate with a fluorometric microplate reader with excitation in the 530-570 nm range and emission in the 590-600 nm range.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Amyloglucosidase (-AG) from the sample well values containing enzyme (+AG) to obtain the difference. The fluorescence difference is due to the Amyloglucosidase activity:

$$\text{Net RFU} = (\text{RFU}_{+\text{AG}}) - (\text{RFU}_{-\text{AG}})$$

5. Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of Starch present in the sample. Only use values within the range of the standard curve.

Example of Results

The following figures demonstrate typical Starch Assay Kit (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

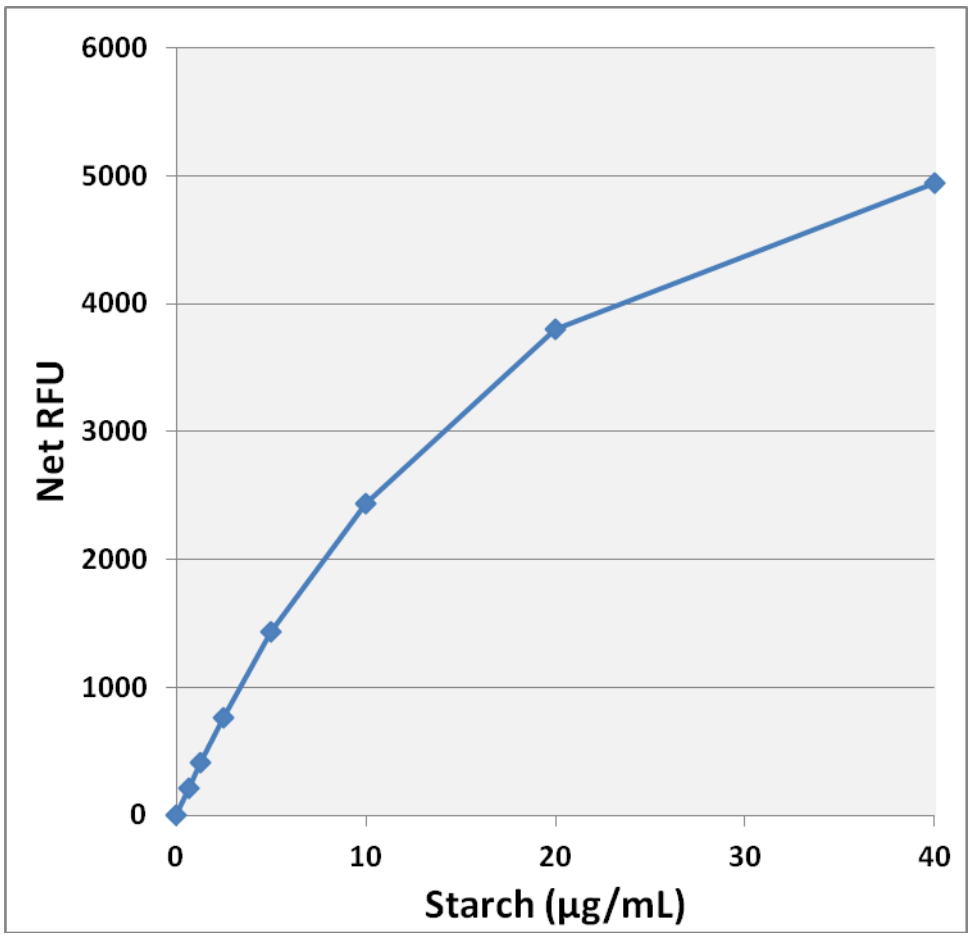

Figure 2: Starch Standard Curve.

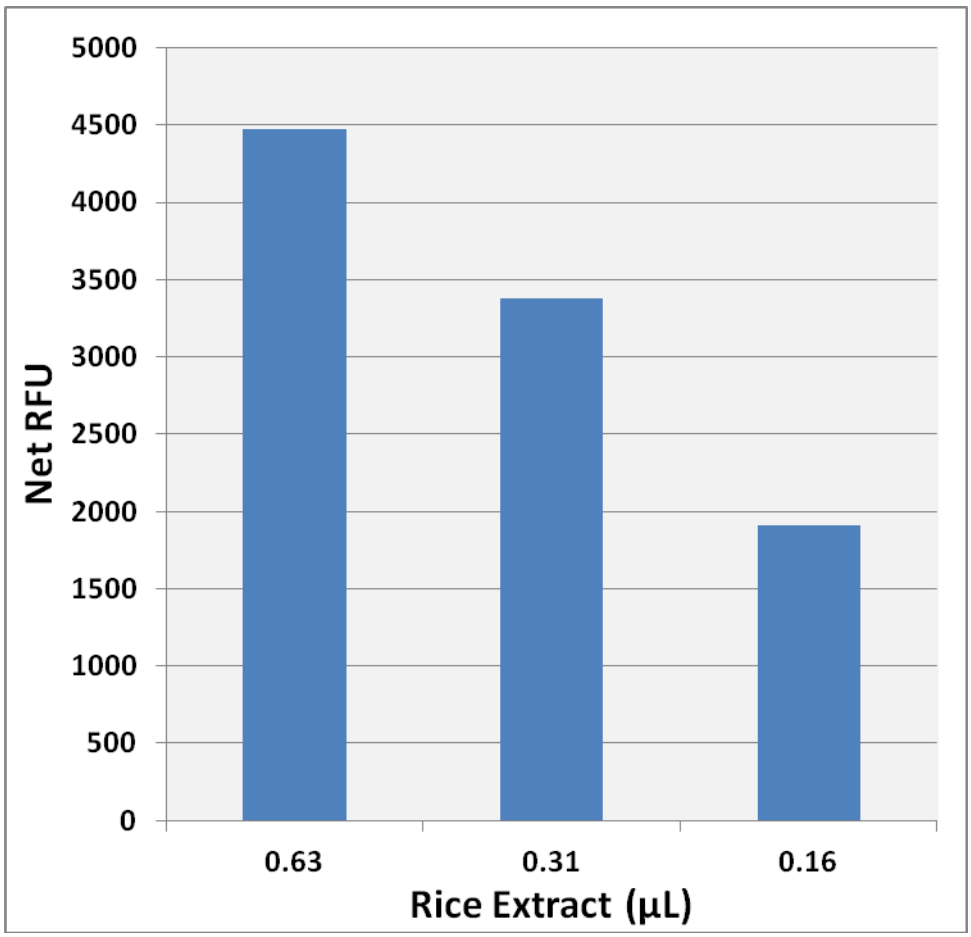

Figure 3: Starch Detection in Total Rice Extract using the Starch Assay Kit (Fluorometric).

References

1. Meekins DA, Vander Kooi CW, and Gentry MS (2016) *FEBS J.* doi:10.1111/febs.13703
2. Votyakova TV and Reynolds IJ (2001) *Neurochem*. **79**: 266.
3. Ačkar D, Babić J, Jozinović A, Miličević B, Jokić S, Miličević, Rajič M, and Šubarić S (2015) *Molecules.* **20**:19554.
4. Tian J, Chen J, Ye X and Chen S (2016) *Food Chem*. **202**:165.
5. Tetlow IJ and Emes MJ (2014) *IUBMB Life.* **66**:546.

Warranty

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