Starch Assay Kit

Starch Assay Kit is a detection kit for the quantification of Starch Content in tissue extracts and cell lysate.

Catalog number: ARG82019

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

Starch or amylum is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants for energy storage. It is the most common carbohydrate in human diets and is contained in large amounts in staple foods like potatoes, maize (corn), rice, wheat and cassava (manioc).

Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight. Glycogen, the glucose store of animals, is a more highly branched version of amylopectin.

In industry, starch is converted into sugars, for example by malting, and fermented to produce ethanol in the manufacture of beer, whisky and biofuel. It is processed to produce many of the sugars used in processed foods. Mixing most starches in warm water produces a paste, such as wheatpaste, which can be used as a thickening, stiffening or gluing agent. The biggest industrial non-food use of starch is as an adhesive in the papermaking process. Starch can be applied to parts of some garments before ironing, to stiffen them. [Provide by Wikipedia: Starch]

PRINCIPLE OF THE ASSAY

This Starch Assay Kit is a simple colorimetric assay that measures the amount of Starch present in tissue extracts and cell lysate. The assay is based on the enzyme driven reaction. 80% ethanol can be used to separate the soluble sugar and starch in the sample. And then decompose starch to glucose. The glucose content can be determined by anthrone colorimetry method, which can calculate starch content. The concentration of Starch in the samples is then determined by comparing the O.D. 620 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well microplate	1 plate	RT
Assay Buffer	4 x 30 mL (ready to use)	4°C
Reaction Buffer	6 mL (ready to use)	4°C
Dye Reagent Diluent	15 mL (ready to use)	4°C
Dye Reagent (lyophilized)	1 vial	4°C
Standards (lyophilized)	1 vial	4°C
Plate sealer	3 ea	RT
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 620 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Concentrated sulfuric acid
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, transfer it to the microcentrifuge tube, and put it in 80°C water bath for 30 minutes, centrifuged at 3,000 x g for 5 minutes at room temperature. Discard the supernatant. Add 1 mL of distilled water into the precipitate, then put it to the boiling water bath for 15 minutes (fasten down, in case moisture loss).

REAGENT PREPARATION

- Dye Reagent: Add 15 mL of Dye Reagent Diluent into the bottle, dissolve it absolutely before use. Store at 4°C.
- Standards: Add 1 mL of distilled water to dissolve before use, mix well, and heat in boiling water bath for 1 minute. The concentration well be 1 mg/mL. Use the 1 mg/mL Standards to prepare a series of diluted standards according to the Table below.

Standard tube	Final Standard conc. (mg/mL)	distilled water (µL)	Volume of 1 mg/mL Standards (µL)
S1	1	0	500
S2	0.5	250	250 of S1
S3	0.25	250	250 of S2
S4	0.125	250	250 of S3
S5	0.0625	250	250 of S4
S6	0.0313	250	250 of S5
S0	0	250	0

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Add following reagents into the microcentrifuge tubes:			
Reagent	**Sample**	**Standards**	**Blank**
Sample	90 µL		
Standards		90 µL	
Reaction Buffer	60 µL	60 µL	
Incubate for **15 minutes** at **room temperature**. Vortex **3-5 times**, then centrifuged at **4,000 x g** for **10 minutes** at **room temperature**.			
Add following reagents into the 96 Well microplate:			
Supernatant	50 µL	50 µL	
Distilled water			50 µL
Dye Reagent	150 µL	150 µL	150 µL
Mix well, and incubate for **15 minutes** at **90°C** in the oven. Read the absorbance at O.D. 620 nm.			

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. According to the weight of sample:

Starch (mg/g)

$= [(C_{Standaard} \times V_{Standardl}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Total})$

$= [(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / W$

Note:

$C_{Protein}$: the protein concentration of sample, mg/mL;

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 1 mg/mL;

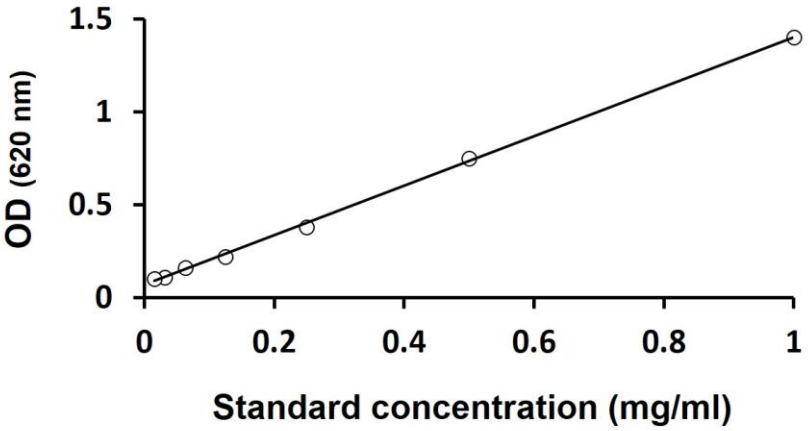
V_{Total}: the total volume of sample, 1 mL;

V_{Sample}: the volume of sample, 0.09 mL;

$V_{standard}$: the volume of Standard, 0.09 mL.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Starch Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.01 mg/mL