ab83393

Starch Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Starch levels in various samples.
View kit datasheet: www.abcam.com/ab83393
(use www.abcam.cn/ab83393 for China, or www.abcam.co.jp/ab83393 for Japan)

This product is for research use only and is not intended for diagnostic use.

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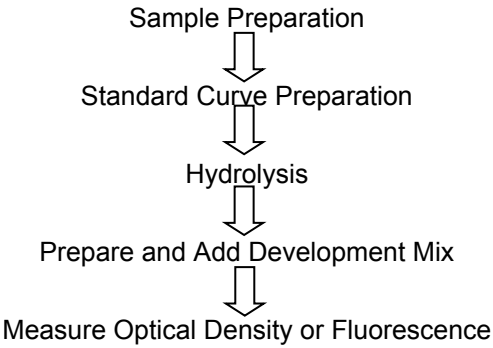
1. Overview

Starch is a complex carbohydrate consisting of a large number of glucose units. All plants contain starch, present as amylose, (linear α-1,4 linked polymer) and amylopectin, (highly α-1,6 branched α-1,4 polymer). Starch generally contains 0-25% amylose and 75-100% amylopectin.

Abcam's Starch Assay Kit provides an easy, accurate assay to measure starch levels in a variety of samples. In the assay, starch is hydrolyzed to glucose which is oxidized to generate color (λ_{max} 570 nm) and fluorescence (Ex/Em = 535/587 nm). The assay can detect starch at 0.0004 to 2 mg/ml.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Hydrolysis Buffer	25 mL
Development Buffer	25 mL
OxiRed Probe	0.4 mL
Hydrolysis Enzyme Mix (Lyophilized)	1 vial
Development Enzyme Mix (Lyophilized)	1 vial
Starch Standard (2.0 mg/ml)	100 µL

* Store kit at -20°C, protect from light and moisture. Warm Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening. Read entire protocol before the assay.

OxiRed PROBE: Ready to use as supplied. Warm up >18°C to melt frozen DMSO before use. Mix well, store at -20°C, protect from light and moisture.

HYDROLYSIS ENZYME MIX, DEVELOPMENT ENZYME MIX: Dissolve with 220 µl Hydrolysis Buffer. Vortex gently to dissolve. Keep on ice. Store at -20°C. Stable for at least two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader
- 96-well plate
- Orbital shaker
- 90% Ethanol
- 10N KOH
- 10M H_3PO_4

4. Assay Protocol

1. Sample Preparation:

Depending on your assay purpose (quantitation, mw distribution, compartmentalization, etc.), prepare starch samples according to established protocols[1-4].

A. Soluble Starch Extraction:

a) Grind up 5-10 mg sample; wash off any free glucose and small oligosaccharides with 1 ml 90% ethanol, warm to 60°C for 5 minutes with occasional vortexing.

b) Centrifuge at 10,000 x g for 2 minutes.

c) Decant the supernatant. Repeat the wash twice. Soluble starch can be extracted with 1 ml H_2O and heating on a boiling water bath for 5 minutes.

d) Spin at 10,000 x g for 2 minutes to remove insoluble materials. The supernatant is soluble starch.

B. Resistant Starch Extraction:

a) After extracting soluble starch, extract the water insoluble pellet with 1 ml 10N KOH, heat on boiling water bath for 5 minutes.

b) Neutralize with 1 ml 10M H_3PO_4 slowly.

c) Spin at 10,000 x g for 2 minutes to remove insoluble materials. The supernatant is resistant starch.

C. Total Starch Extraction:

After the 90% ethanol wash (Step A), extract the washed sample directly with 10N KOH/H_3PO_4 as per the procedure for resistant starch (B). The supernatant is total starch.

For starch sample testing: Take 20 µl of the extracted starch, add 180 µl of Hydrolysis Buffer, mix.

Add up to 50 µl of the diluted sample or buffer (blank) to test wells. Adjust the volume to 50 µl with Hydrolysis Buffer.

For unknown samples, we suggest testing several doses of the sample to ensure the readings are within the standard curve.

2. Standard Curve Preparation:

a. For the colorimetric assay: Dilute Starch Standard to 0.2 mg/ml by adding 10 µl of the Standard to 90 µl of distilled water, mix well. Add 0, 2, 4, 6, 8, 10µl into a series of wells.

Adjust volume to 50 µl/ well with Hydrolysis Buffer to generate 0, 0.4, 0.8, 1.2, 1.6 and 2.0 µg/well of the Starch Standard.

b. For the fluorometric assay: Dilute Starch Standard to 0.02 mg/ml by adding 10 µl of the Standard to 990 µl of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells.

Adjust volume to 50 µl/well with Hydrolysis Buffer to generate 0, 0.04, 0.08, 0.12, 0.16 and 0.2 µg/well of Starch Standard.

3. Hydrolysis*:	**Colorimetric**	**Fluorometric**
Hydrolysis Enzyme Mix	2 µl	1 µl

Mix well. Incubate for at least 30 minutes at room temperature to hydrolyze starch.

* **Note:** Glucose generates background. Glucose control is done without the hydrolysis enzyme (add equal volume of Buffer). Glucose background can be subtracted from sample reading.

4. **Development:** Mix enough reagents for the number of samples and standards. For each well, prepare a total 50 µl Reaction Mix.

	Colorimetric	**Fluorometric**
Development Buffer	46 µl	48.7 µl
Development Enzyme Mix	2 µl	1 µl
OxiRed Probe	2 µl	0.3 µl

Add 50 µl of Development Mix to each well containing Starch Standard or samples. Incubate at room temperature for 30 minutes, protect from light.

5. **Measurement:** Measure colorimetrically (OD_{570nm}) or fluorometrically (Ex/Em = 535/587 nm).

5. Data Analysis

Correct background by subtracting the value of the zero starch control from all sample readings. The background can be significant and must be subtracted). Plot standard curve µg/well vs OD.

Apply sample readings to the standard curve to get the amount of starch in the sample wells. The starch concentration in the test samples:

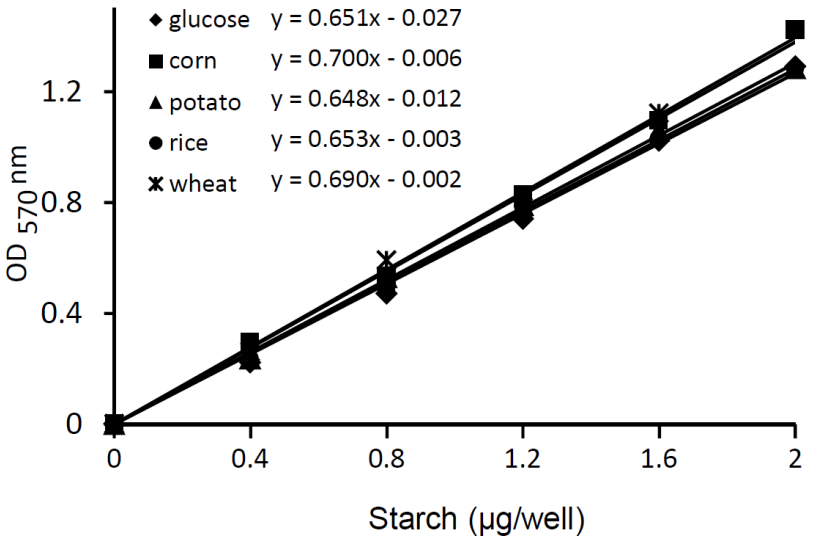
Concentration = Ay / Sv (µg/µl, or mg/ml)

Where:

Ay is the amount of starch (µg) in your sample from the standard curve.

Sv is the sample volume (µl) added to the sample well. Multiply by the dilution factors.

Starch molecular size: ~60,000 glucose molecules (MW ~10^6-10^7daltons).



Starch Standard Curve: Different types of pure starch were extracted with 10N KOH/H_3PO_4 as described following the kit protocol.

6. References

1) Quantitative Isolation and Dispersion of Starch from Corn Kernels without Degradation, James P. McGuire, Stig R. Erlander, Starch-Stärke, Vol 18, No. 11, (2006) 342-346.

2) Overview of Laboratory Isolation of Starch from Plant Materials, Thava Vasanthan, Current Protocols in Food Analytical Chemistry, UNIT E2.1 (2001), John Wiley & Sons, Inc.

3) A rapid micro-starch quantitation method for potato callus and its application with potato tubers, J. L. Varns and J. R. Sowokinos, Journal American Journal of Potato Research Vol. 51, No. 12(1974).

4) Critical study of a procedure for the assay of starch in ligneous plants, Gomez, L., Rubio E., Lescourret F., Journal of the Science of Food and Agriculture, Vol. 83, No. 11 (2003) 1114-1123.

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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