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Product Information

Starch Colorimetric/Fluorometric Assay Kit

Catalog Number **MAK368**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Starch is a complex carbohydrate consisting of a large number of glucose units. All plants contain starch, present as amylose, (linear $\alpha(1\rightarrow4)$ linked polymer) and amylopectin, (highly $\alpha(1\rightarrow6)$ branched $\alpha(1\rightarrow4)$ polymer). Starch generally contains 0–25% amylose and 75–100% amylopectin.

The Starch Assay Kit provides an easy, convenient method to measure starch levels in a variety of samples. In the assay, starch is hydrolyzed to glucose which is oxidized to generate color at 570 nm (A_{570}) and fluorescence (λ_{ex} = 535 nm/λ_{em} = 587 nm). The assay can detect starch at 0.0004 to 2 mg/mL.

The kit is suitable for the measurement of starch in a variety of samples.

Components

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

Hydrolysis Buffer Catalog Number MAK368A	25 mL
Development Buffer Catalog Number MAK368B	25 mL
OxiRed Probe Catalog Number MAK368C	0.4 mL
Hydrolysis Enzyme Mix Catalog Number MAK368D	1 vial
Development Enzyme Mix Catalog Number MAK368E	1 vial
Starch Standard (2.0 mg/mL) Catalog Number MAK368F	100 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- Microcentrifuge capable of RCF ≥10,000 × *g*
- Ethanol, 200 Proof (Catalog Number E7023)
- 10 M Potassium Hydroxide Solution
- Concentrated Phosphoric Acid (Catalog Number 438081)

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, protected from light and moisture. Warm Buffers to room temperature prior to use. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

OxiRed Probe: Ready to use as supplied. Prior to use, warm solution to >18 °C to melt frozen DMSO, mix well.

Hydrolysis Enzyme Mix and Development Enzyme Mix: Dissolve each vial with 220 µL of Hydrolysis Buffer. Vortex gently to dissolve. Keep on ice while in use. Stable for at least two months once reconstituted.

Starch Standard: Prior to use, heat the starch standard to 100 °C for 5 minutes in a heat block or boiling water. After heating, vortex contents for 5 seconds to dissolve any precipitate.

90% Ethanol: For each mL needed, add 900 μL of 200 proof Ethanol to 100 μL of ultrapure water.

10 M Phosphoric Acid (H_3PO_4): Add 68.424 mL of concentrated phosphoric acid to 25 mL of ultrapure water. Adjust the final volume to 100 mL with ultrapure water, mix well.

Procedure

Sample Preparation

Depending on the assay purpose (quantitation, mw distribution, compartmentalization, etc.), prepare starch samples according to established protocols.[1,2,3,4]

1. Soluble Starch Extraction
   a. Grind 5–10 mg of sample.
   b. Wash off any free glucose and small oligosaccharides with 1 mL of 90% ethanol.
   c. Warm to 60 °C for 5 minutes with occasional vortexing.
   d. Centrifuge at 10,000 × *g* for 2 minutes.
   e. Decant the supernatant.
   f. Repeat the 90 % ethanol wash twice.
   g. Soluble starch can be extracted with 1 mL ultrapure water and heating on a boiling water bath for 5 minutes.
   h. Centrifuge at 10,000 × *g* for 2 minutes to remove insoluble materials. The supernatant is soluble starch.

2. Resistant Starch Extraction
   a. After extracting soluble starch, extract the water insoluble pellet with 1 mL of 10 M KOH.
   b. Heat in a boiling water bath for 5 minutes.
   c. Add 1 mL of 10 M H_3PO_4 slowly.
   d. Centrifuge at 10,000 × *g* for 2 minutes to remove insoluble materials. The supernatant is resistant starch.

3. Total Starch Extraction
   a. Grind 5–10 mg of sample.
   b. Wash off any free glucose and small oligosaccharides with 1 mL of 90% ethanol.
   c. Extract the washed sample directly with 10 M KOH/10 M H_3PO_4 as per the procedure for resistant starch (Preparation 2).
   d. 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, test several levels of the sample to ensure the readings are within the standard curve.

Colorimetric Standard Curve Preparation

Prepare a 0.2 mg/mL Starch Standard by adding 10 μL of the Starch Standard (2.0 mg/mL) to 90 μL of ultrapure water, mix well. Prepare Starch Standards in desired wells of a clear flat-bottom 96 well plate according to Table 1.

Table 1.
Preparation of Starch Standards (Colorimetric)

Well	0.2 mg/mL Premix	Hydrolysis Buffer	Starch (μg/well)
1	0 μL	50 μL	0
2	2 μL	48 μL	0.4
3	4 μL	46 μL	0.8
4	6 μL	44 μL	1.2
5	8 μL	42 μL	1.6
6	10 μL	40 μL	2.0

Fluorometric Standard Curve Preparation

Prepare a 0.02 mg/mL Starch Standard by adding 10 μL of the Starch Standard (2.0 mg/mL) to 990 μL of ultrapure water, mix well. Prepare Starch Standards in desired wells of a black flat-bottom 96 well plate according to Table 2.

Table 2.
Preparation of Starch Standards (Fluorometric)

Well	0.02 mg/mL Premix	Hydrolysis Buffer	Starch (μg/well)
1	0 μL	50 μL	0
2	2 μL	48 μL	0.04
3	4 μL	46 μL	0.08
4	6 μL	44 μL	0.12
5	8 μL	42 μL	0.16
6	10 μL	40 μL	0.20

Hydrolysis

To all sample and standard wells, add 2 μL (colorimetric assay) or 1 μL (fluorometric assay) of Hydrolysis Enzyme Mix. Mix well and incubate for at least 30 minutes at room temperature to hydrolyze starch.

Glucose Background Control
The presence of glucose in samples will generate background. For samples that may contain glucose, prepare a glucose control. To a separate well, add the same volume of sample that was added to the sample well. Adjust the volume with Hydrolysis Buffer to 52 µL (colorimetric assay) or 51 µL (fluorometric assay). Do not add Hydrolysis Enzyme Mix.

Development Reaction Mix
Mix enough reagents for the number of samples, glucose background controls (if applicable), and standards. For each well, prepare a total of 50 µL of Development Reaction Mix according to Table 3.

Table 3.
Preparation of Development Reaction Mix

Reagent	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.

Measurement
Incubate plate(s) for 30 minutes at room temperature, protected from light. Measure colorimetrically (A_{570}) or fluorometrically (λ_{ex} = 535 nm/λ_{em} = 587 nm).

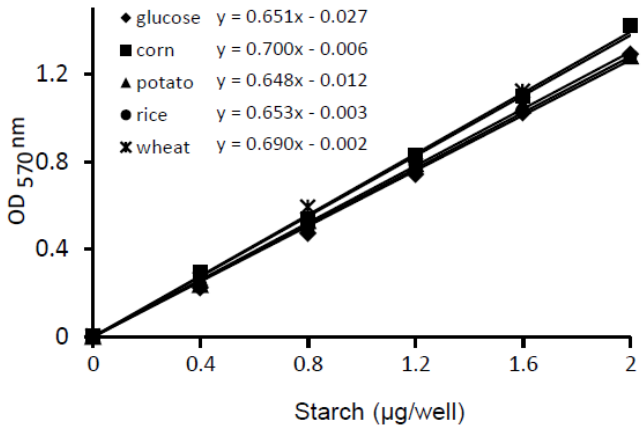
Results
1. Correct background by subtracting the value of the 0 starch standard from all sample readings.
   Note: The background can be significant and must be subtracted.
2. Plot the standard curve µg/well vs. A_{570} or FLU (λ_{ex} = 535 nm/λ_{em} = 587 nm).
3. If a glucose background control was run, subtract the value of the control from the corresponding sample value.
4. Compare sample readings to the standard curve to obtain the amount of starch in the sample wells.
5. Calculate the starch concentration in the test samples:

   Starch (µg/µL or mg/mL) = (Ay/Sv) × D

where:
Ay = the amount of starch (µg) in the sample from the standard curve
Sv = the volume of sample added to the reaction well (µL)
D = the sample dilution factor

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

Figure 1.
Typical Starch Standard Curve



Different types of pure starch were extracted with 10 M KOH/H_3PO_4 as described following the kit procedure.

References

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3. J. L. Varns and J. R. Sowokinos, A rapid micro-starch quantitation method for potato callus and its application with potato tubers. Journal American Potato, **51**(12), 383-392 (1974).
4. Gomez, L. et al., Critical study of a procedure for the assay of starch in ligneous plants, Journal of the Science of Food and Agriculture, **83**(11), 1114-1123 (2003).

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