Amylose/Amylopectin Assay Kit

Kit-2414
Lot. No. (See product label)

Specification

Product Overview	The Amylose/Amylopectin test kit is suitable for the measurement and analysis of amylose/amylopectin ratio and content in cereal starches and flours. Based on a Con A precipitation procedure.
Notes	Starch samples must be pre-treated with ethanol as described to remove lipids. If samples are not treated with ethanol, the amylose contents in some samples may be under-estimated by as much as 50%.
Size	100 assays
Kit Components	Bottle 1: Freeze dried Con A. Stable for > 5 years at -20°C. Bottle 2: Amyloglucosidase [200 U on p-nitrophenyl β-maltoside (i.e. 3,300 U on starch at pH 4.5 at 40°C)] plus fungal α-amylase (500 U on Ceralpha Reagent at pH 5.0 and 40°C), 2 mL. Stable for > 5 years at 4°C. Bottle 3: GOPOD Reagent Buffer. Buffer (50 mL, pH 7.4), p-hydroxybenzoic acid and sodium azide (0.095% w/v). Stable for > 4 years at 4°C. Bottle 4: GOPOD Reagent Enzymes. Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze dried powder. Stable for > 5 years at -20°C. Bottle 5: D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid. Stable for > 5 years at room temperature. Bottle 6: Starch reference sample (with specified content of amylose). Stable for > 5 years at room temperature.
Materials Required but Not Supplied	BUFFERS AND SOLVENTS (NOT SUPPLIED): 1. Sodium Acetate Buffer (100 mM, pH 4.5) Add 5.9 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH to pH 4.5 by the addition of 1 M (4 g/100 mL) sodium hydroxide solution (approx. 30 mL is required). Add 0.2 g of sodium azide and adjust the volume to 1 L. Stable for > 2 years at room temperature. 2. Concentrated Con A Solvent (600 mM, pH 6.4 sodium acetate buffer) Dissolve 49.2 g of anhydrous sodium acetate (Sigma cat. no. 71183), 175.5 g of sodium chloride (Sigma cat. no. S7653), 0.5 g of CaCl2.2H2O (Sigma cat. no. C5080), 0.7 g of MgCl2.6H2O (Sigma cat. no. M2670) and 0.7 g of MnCl2.4H2O (Sigma cat. no. M3634) in 900 mL of distilled water. Adjust the pH to 6.4 by dropwise addition of glacial acetic acid and then adjust the volume to 1 L with distilled water. Stable for 2 weeks at 4°C. 3. Con A Solvent (working concentration) Dilute 30 mL of Concentrated Con A Solvent to 100 mL with distilled water. Use on the day of preparation.

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4. Dimethyl sulphoxide (DMSO)
Analytical reagent grade (BDH Analar cat. no. 10323).
Stable for 5 years at room temperature.

EQUIPMENT (RECOMMENDED):
1. Glassware:
- volumetric flask (25 mL);
- glass test tubes (16 x 120 mm, 15 mL);
- screw capped sample tubes (Kimax®)(10 mL).
2. Micro-pipettors, to dispense 50-1000 µL (e.g. Gilson Pipetman).
3. Positive displacement pipettor, e.g. Eppendorf Multipette®.
4. Eppendorf® microfuge tubes (2.0 mL capacity).
5. Boiling water bath.
6. Bench centrifuge (capable of 2,000 g).
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
8. Spectrophotometer (set at 510 nm).
9. Stop clock.
10. Analytical balance.
11. Microfuge (capable of 14,000 g).
12. Thermostated water bath set at 40°C.

Detection method	Spectrophotometric at 510 nm

Preparation

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:
1. Dissolve the contents of bottle 1 in 50 mL of Con A solvent (Buffer 3). Divide into aliquots of appropriate size and store in polypropylene tubes at -20°C between use and keep cool during use if possible. Stable for > 2 years at -20°C.
2. Dissolve the contents of bottle 2 in 20 mL of sodium acetate buffer (100 mM, pH 4.5). Divide into appropriately sized aliquots and store in polypropylene tubes at -20°C between use and keep cool during use if possible. Stable for > 2 years at -20°C.
3. Dilute the contents of bottle 3 (GOPOD Reagent Buffer) to 1 L with distilled water (this is solution 3). Use immediately.

NOTE:
1. On storage, salt crystals may form in the concentrated buffer. These must be completely dissolved when this buffer is diluted to 1 L with distilled water.
2. This buffer contains 0.095% (w/v) sodium azide. This is a poisonous chemical and should be treated accordingly.

4. Dissolve the contents of bottle 4 with 20 mL of solution 3 and quantitatively transfer this to the bottle containing the remainder of solution 3. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is Glucose Determination Reagent (GOPOD Reagent). Stable for ~ 3 months when stored at 2-5°C or > 12 months at -20°C.
5 & 6. Use the contents of bottles 5 and 6 as supplied. Stable for > 5 years at room temperature.

SAFETY CONSIDERATIONS:

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1. Dimethyl sulphoxide (DMSO) is listed in the Merck Index (No. 3255) as a skin irritant and thus it should be used with caution. It is absorbed through the skin and can cause irritation to both skin and eyes. Wear PPE and avoid splashing the solvent. Use in a fume cupboard where possible.
2. Concanavalin A is harmful by inhalation, skin contact and ingestion. Effects may be irreversible and may involve teratogenesis. Wear appropriate PPE when handling crystalline Con A and gloves when handling solutions containing Con A.
3. Sodium azide is a toxic chemical and should be treated accordingly. It is added to buffers solely as a preservative. It can be deleted from buffer recipes but buffers should then be stored at 4°C.

Assay Protocol

A. Starch Pre-treatment
1. Accurately weigh starch or flour sample (20-25 mg to the nearest 0.1 mg) into a 10 mL screw capped Kimax® sample tube. Record the sample weight to the nearest 0.1 mg.
NOTE: Include a reference sample with each batch. Duplicate every fifth test sample.
2. Add 1 mL of DMSO to the tube while gently stirring it at low speed on a vortex mixer. Cap the tube and heat the tube contents in a boiling water bath until the sample is completely dispersed (approx. 1 min). Ensure that no gelatinous lumps of starch are remaining.
3. Vigorously mix the contents of the sealed tube at high speed on a vortex mixer, place the tube in a boiling water bath and heat it for 15 min, with intermittent high-speed stirring on a vortex mixer.
4. Store the tube at room temperature for approx. 5 min and add 2 mL of 95% (v/v) ethanol with continuous stirring on a vortex mixer. Add a further 4 mL of ethanol, cap the tube and invert to mix. A starch precipitate will form. Allow the tube to stand for 15 min (or overnight if desired).
5. Centrifuge the tubes at 2,000 g for 5 min, discard the supernatant and drain the tubes on tissue paper for 10 min. Ensure that all of the ethanol has drained. Use the pellet in the subsequent amylose and starch determinations.
6. Add 2 mL of DMSO (with gentle vortex mixing) to the starch pellet. Place the tube in a boiling water bath for 15 min and mix occasionally. Ensure that there are no gelatinous lumps.
7. On removing the tubes from the boiling water bath, immediately add 4 mL of Con A solvent (Buffer 3; page 4), mix thoroughly and then quantitatively transfer the tube contents (by repeated washing with Con A solvent) to a 25 mL volumetric flask. Dilute to volume with Con A solvent (this is Solution A). If necessary, filter this solution through Whatman® No. 1 filter paper (this step will be necessary for whole flour samples).
NOTE: This solution should be analysed within 2 h.

B. Con A Precipitation of Amylopectin and Determination of Amylose
1. Transfer 1.0 mL of Solution A to a 2.0 mL Eppendorf® microfuge tube. Add 0.50 mL of Con A solution (bottle 1), cap the tube and gently mix by repeated inversion. Avoid frothing of the sample.
2. Allow the tube to stand for 1 h at room temperature. Centrifuge at 14,000 g for 10 min in a microfuge at room temperature.

NOTE:
1. Samples in Con A Solvent (i.e. Solution A as described in Section A above) cannot be left for extended periods because the amylose will tend to retrograde and precipitate.
2. The time required for effective Con A precipitation of the amylopectin (Step B1 above) is 1 h at

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	room temperature. However, these solutions should not be left for longer than 2 h as the amylose will tend to retrograde. 3. In this procedure, pre-treatment of the samples with ethanol has the added advantage of removing any soluble sugars in the sample that would otherwise interfere with the assay. 3. Transfer 1 mL of the supernatant to a 15 mL centrifuge tube. Add 3 mL of 100 mM sodium acetate buffer, pH 4.5. This reduces the pH to ~ 5. Mix the contents, lightly stopper (with a marble) and heat in a boiling water bath for 5 min to denature the Con A. 4. Place the tube in a water bath at 40°C and allow to equilibrate for 5 min. Add 0.1 mL of amyloglucosidase/α-amylase enzyme mixture (page 3; solution 2) and incubate at 40°C for 30 min. Centrifuge the tube at 2,000 g for 5 min. 5. To 1.0 mL aliquots of the supernatant add 4 mL of GOPOD Reagent (Reagent B). Incubate at 40°C for 20 min. Incubate the Reagent Blank and the D-Glucose Controls concurrently. NOTE: The Reagent Blank is prepared by adding 1.0 mL of 100 mM sodium acetate buffer (Buffer 1; page 4) to 4.0 mL of GOPOD Reagent and incubating at 40°C for 20 min. D-Glucose Controls (duplicate) comprise 0.1 mL of D-glucose standard solution (1 mg/mL), 0.9 mL of sodium acetate buffer and 4.0 mL of GOPOD Reagent. This value is not used in the calculation, however we suggest that it is performed to ensure that there are no problems with this part of the assay. 6. Read the absorbance of each sample and the D-glucose controls at 510 nm against the reagent blank. C. Determination of Total Starch 1. Mix 0.5 mL of Solution A with 4 mL of 100 mM sodium acetate buffer, pH 4.5. 2. Add 0.1 mL of amyloglucosidase/α-amylase solution and incubate the mixture at 40°C for 10 min. 3. Transfer 1.0 mL aliquots (in duplicate) of this solution to glass test tubes, add 4 mL of GOPOD Reagent (solution 4) and mix well. Incubate at 40°C for 20 min. This incubation should be performed concurrently with the samples and standards from Section B above.
Assay time	~ 120 min
Analysis	CALCULATION OF AMYLOSE CONTENT (%): Amylose, % (w/w) = [Absorbance (Con A Supernatant)/ Absorbance (Total Starch Aliquot)]x (6.15/9.2) x (100/1)= [Absorbance (Con A Supernatant)/ Absorbance (Total Starch Aliquot)] x 66.8 Where 6.15 and 9.2 are dilution factors for the Con A and Total Starch extracts respectively.
Sensitivity	Amylose 5-95% of total starch content

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