Megazyme Starch Procedures
Updated 9-12-13 NAA

Reference
Megazyme Total Starch Assay: www.megazyme.com

Supplies
Vortex
100^{o}C water bath
50^{o}C water bath

Personal Protective Equipment
1. Lab coat
2. Safety glasses/goggles
3. Latex gloves

Reagents
80% v/v aqueous ethanol

1 M NaOH
4 g NaOH pellets/ 100 mL ddH_2O

100mM Sodium Acetate Buffer-
In 1 L volumetric
900 mL ddH_2O + 5.8 mL glacial acetic acid
Adjust to pH 5.0 w/ 1 M NaOH (~30 mL)
Add 0.74 g CaCl 2H_2O
q.s. to 1 L
Store at 4^{0}C

α-amylase solution
In a 100 mL volumetric flask
Pipette 3.333 mL a-amylase from bottle in kit
q.s. to 100 mL with **100mM Sodium Acetate Buffer** solution
Store at 4^{o}C

Amyloglucosidase- Store at 4^{o}C
bottle in kit

GOPOD- store at 4^{o}C stable up to 6 months
In 1 L volumetric
Contents of GOPOD Reagent **Buffer** bottle from kit
q.s. to 1 L with ddH_2O
Transfer to aluminum foil covered bottle
Put 20 mL of this solution in GOPOD Reagent **Enzyme** bottle from kit
Transfer all of the 20 mL back to the aluminum covered bottle

Reagents below for KOH Procedure ONLY:

2 M Potassium Hydroxide solution (KOH)

In 1 L volumetric
112.2 g KOH to 900 mL ddH_2O and stir to dissolve
q.s. to 1 L w/ ddH_2O
Store in sealed container

4 M Sodium Hydroxide (NaOH)

16 g NaOH pellets/ 100 mL ddH_2O

1.2 M Sodium Acetate Buffer

In 1 L volumetric
800 mL ddH_2O + 69.6 mL glacial acetic acid
Adjust to pH 3.8 using 4 M NaOH
q.s. to 1 L

Blank, Control, and Standards

Blank

0.1 mL ddH_2O in small glass tubes with 3 mL GOPOD (in triplicate)

Control

0.1 mL glucose standard from kit bottle with 3 mL GOPOD (in triplicate)

Standards: use corn starch

Starch%	NEEDED g starch/ 100 mL
0	0.0
15	0.0167
30	0.0333
45	0.0500
60	0.0666
75	0.0832

*Include DRC standard using 0.05 g treat DRC the same as unknown samples.

Note: When creating new starch standards, in order to calculate the correct amount of glucose to weigh up, take the amount of starch needed and divide by 0.9 to get the actual amount of glucose needed for the procedure.

Pipette 0.1 mL of each standard (in triplicate) into little glass tubes.

Incubate all at 50^{o}C for 20 min

Calculations from Megazyme
Starch, % DM = A * F/W * FV * 0.9 * (100/ (100-% moisture content))

A = Absorbance read against the reagent blank

F = 100/ absorbance for control

W = weight of sample in mg (as-is)

FV = final volume (100; if 100 mL volumetric flasks were used)

Note: Spectrophotometer output is total mg of starch if 100mL volumetric flasks were used.

*<u>No resistant starch w/ incubations at pH 5.0</u>

1. Samples weighed into 16x120 mm tubes in triplicate. Prepare standards in the same manner.
   NOTE:

Sample Type	Sample Weight
Corn	0.05 g
Fecal	0.15 g
Duodenal-Diet-Orts	0.10 g

2. Add 0.2 mL 80% ethanol to each tube and vortex.
3. Immediately add 3 mL dilute α-amylase and vortex.
4. Incubate tubes in 100^{o}C water bath 2 min. and vortex vigorously (no lumps).
5. Incubate 2 more minutes and vortex vigorously (no lumps).
6. Incubate 2 more minutes and vortex vigorously (no lumps).
7. Place tubes in 50^{o}C water bath.
8. Add 0.1 mL amyloglucosidase.
9. Stir with vortex and incubate at 50^{o} C for 30 min.
10. Transfer contents of the tube to 100 mL flask w/ funnel:
    - rinse tubes well with ddH_2O to ensure no residue remains
    - q.s. flask to 100 mL and shake well
11. Centrifuge an aliquot of this solution at 1,800 *g* for 10 minutes.
12. Transfer (in duplicate) 0.1 mL of the solution to little glass tubes.
13. Add 3 mL GOPOD and vortex lightly.
14. Incubate tubes at 50^{o} C for 20 minutes.
15. Vortex lightly and pipette 300 µL from each glass tube into 96 well plate.
16. Read on spec at 510 nm.

KOH procedure for resistant starch

1. Samples weighed into 16x120 mm tubes in triplicate in plastic rack.
2. Add 0.2 mL 80% ethanol to each tube and vortex.
3. Add stir bar to each tube.
4. Place in a plastic butter dish packed with ice water on stir plate and start stirring vigorously: (it is a good idea to label tubes before putting on ice and tape the tubes together so the tubes remain upright).
5. Add 2 mL 2 M KOH.
6. Stir vigorously for 20 minutes.
7. Continue stirring, and add 8 mL of 1.2 M Sodium acetate buffer to each tube.
8. Immediately add 0.1 mL α-amylase and 0.1 mL amyloglucosidase (**Both directly from the kit bottles with a multipipeter/repeater)**, mix well, and place in a 50^{o}C water bath.
9. Incubate 10 minutes and vortex.
10. Incubate 10 minutes and vortex.
11. Incubate 10 minutes and vortex, for a total of 30 minutes on in the water bath.
12. While retaining stir bar with an external magnet, Transfer contents of the tube to 100 mL flask w/ funnel:
    - rinse tubes well with ddH_2O to ensure no residue remains
    - q.s. flask to 100 mL and shake well
13. Centrifuge an aliquot of the solution at 1,800 *g* for 10 min.
14. Transfer (in duplicate) 0.1 mL of the solution to little glass tubes.
15. Add 3 mL GOPOD and vortex lightly.
16. Incubate tubes at 50^{o} C for 20 minutes.
17. Vortex lightly and pipette 300 µL from each glass tube into 96 well plate.
18. Read on spec at 510 nm.

Notes:
In future when running some DGS samples run samples through assays with no enzyme addition—use ddH_2O instead pg. 8