

Leaf starch assay

Reagents:

Killing solution

	<u>Volume</u>	<u>Final concentration</u>
Ethanol	160 ml	80% (v/v)
Formic acid	10 ml	5% (v/v)
dH ₂ O	30 ml	15% (v/v)

80% Ethanol

	<u>Volume</u>	<u>Final concentration</u>
Ethanol	160 ml	80% (v/v)
dH ₂ O	40 ml	20% (v/v)

Lugol's IKI solution

Iodine	5.7 mM
Potassium iodide	43.4 mM

It takes a long while for iodine to dissolve in water. Iodine will stain plastics; use a glass beaker to dissolve and use a glass bottle to store the reagent. The reagent is light-sensitive; wrap the bottle with foil and store at 4°.

Harvest:

1. Preheat oven to 80°C.
2. Place a barcode sticker (SA1001) on the side of a microplate.
3. Set up the laptop computer and the barcode scanner. Log onto 2010 Starch Database - Harvest.
4. To begin a harvest, scan the microplate barcode and place the microplate in the CoolSafe system that has been pre-chilled at – 20°C.
5. Take one flat of the Arabidopsis plants from the growth chamber, place under the tabletop fluorescent light fixture (~ 100 µE).
6. Scan the pot barcode, take one leaf disk from leaf number 8 of the plant (counting from the newest visible leaf) with #2 cork borer and place the leaf disk in the designated well in the microplate as shown on the screen.
7. Click the “submit” button on the screen to advance to the next well (alphanumeric order).
8. Repeat step 4-5 for the rest of the plants.
9. Repeat step 3-6 for the other two flats.
10. Scan the microplate barcode to end the harvest.

Processing plate:

1. Pour 25 ml of killing solution into a reservoir. Add 200 µl of killing solution to each well with an 8-channel pipettor.

2. Push leaf disks that are not submerged into the liquid with toothpicks. Seal the plate with aluminum sealing foil and cover with the plate lid.
3. Incubate plate 10 min. at 80°C.
4. Carefully take the plate out of the oven and cool to RT on bench for 1 min.
5. Remove the killing solution.
6. Add 200 µl 80% ethanol to wells and incubate the leaf discs 5 min, 80°C. Remove ethanol.
7. Stain the leaf discs in 200 µl Lugol's IKI solution at RT, 3 min. Remove IKI solution.
8. Rinse leaf discs with 200 µl of distilled H₂O.
9. Add 200 µl of fresh H₂O to each well and make sure the leaf disks lay flat.
10. Incubate the stained plate at 80°C, 15 min, then 4°C, 15 min.

Analyzing starch AM plate:

1. Score individual leaf disc as starch excess or normal on your notebook. Starch-excess mutants stain dark blue with IKI compared to wild-type.
2. Open the images of the plate under filmstrip view, verify your scores by comparing the plate, the images and your lab notebook. Input verified scores (starch excess or normal).

Analyzing starch PM plate:

Same as “**Analyzing starch AM plate**” except that the result of starch assay is either starch-less or normal. Starch-less mutants will not stain dark blue with IKI. They will be yellow or white.

Adapted from:

Yu TS, Kofler H, Hausler RE, Hille D, Flügge UI, Zeeman SC, Smith AM, Kossmann J, Lloyd J, Ritte G, et al (2001) The Arabidopsis *sex1* mutant is defective in the R1 protein, a general regulator of starch degradation in plants, and not in the chloroplast hexose transporter. *Plant Cell* 13:1907-1918.