

ARABIDOPSIS SEED STARCH ASSAY

REAGENTS:

1. 80% ethanol
2. IKI Solution:
iodine, 0.67% (w/v)
potassium iodide, 3.33% (w/v)

PROCEDURE:

1. Scan plate barcode into database.
2. Scan seedstock barcode into database. Tap a fine monolayer of the seed stock into well A1 of a clear polystyrene 96-well microtiter plate. Repeat for each seed stock, following alphanumeric order and database guide.
3. Add 200 ul of 80% ethanol to each well.
4. Seal the microtiter plate with aluminum sealing film and incubate for 20 minutes at 80° C.
5. Cool plate at RT on the benchtop for 2 minutes.
6. Aspirate 80% ethanol from each well.
7. Add 200 ul IKI solution to each well.
8. Incubate the plate at RT for 3 minutes.
9. Aspirate the IKI solution.
10. Wash each well 2X with 200 ul dH₂O by adding and aspirating.
11. After washes, add 200 ul dH₂O to each well. Seeds are photographed and scored while immersed in dH₂O.
12. Photograph the plate immediately. White background, one 100 AC strobe, 60 mm macro lens, F/16, 1/1000 s.

SCORING

Positive = “excess”: blackened seeds
Negative = “normal”: brown or tan seeds

Examine samples with a 10X dissecting microscope to confirm questionable phenotypes.

Reference: Caspar, T., Huber, S.C., and Somerville, C.R. 1985. Alterations in Growth, Photosynthesis, and Respiration in a Starchless Mutant of *Arabidopsis thaliana* (L.) Deficient in Chloroplast Phosphoglucomutase Activity. *Plant Physiol.* 79: 11-17.