Fluorol Yellow Staining (Suberin Staining)

1. Vertically grown (in ½ MS-agar plates) 5-days old seedlings are incubated in a freshly prepared solution of Fluorol Yellow 088 (0.01%w/v, in lactic acid) at 70°C for 30 min.
2. Rinse them in water (three baths of 5 min each).
3. Counter-staining is done with aniline blue (0.5% w/v, in water) at room temperature for 30min in darkness.
4. Washed the samples in water for, at least, 30min (change the bath to fresh water every 10 minutes).
5. Mount on slides using glycerol 50% prior to microscope examination.

Remarks:

- Always use a freshly prepared solution of Fluorol Yellow.
- Use 12-wells microtiter plates for incubations.
- Avoid squeezing roots, use featherweight forceps.

Microscopy and Quantitative Analysis:

Use a wide-field microscope with a standard GFP filter to observe Fluorol Yellow.

Remarks:

- After staining, keep samples in the dark.
- Do not use samples 3 hours after preparation, as the fluorescent signal may leak into the xylem.
- Do not keep the seedling under fluorescence for longer than 20 minutes, as Fluorol Yellow is easily bleached.

Counting:

Wash seedlings several times in water in order to eliminate the counter-staining (Aniline blue). Otherwise, it is impossible to count as counterstaining turns the root dark blue and cells are no longer visible. It is not easy to count cells even after thorough washing. Nevertheless, it is possible to know the borders of cells knowing approximately the average length of endodermal cells. Errors are unavoidable, but with training one can get reproducible results with a reasonable error.

In order to count the endodermal cells, it is easier to go to the point where the Fluorol Yellow signal appears under GFP conditions and switch to bright field or DIC optics to
count the number of cells from this point towards the root tip until the first cell of the elongation zone.

Remarks:

When counting, “Onset of elongation” was defined as the point where endodermal cells in a median optical section are clearly more than twice the width of the previous

cell. The Fluorol Yellow signal initially shows a “patchy” appearance, which at one point turns into a continuous signal, where all endodermal cells are stained. It is better to count both areas, the patchy signal and the onset of a continuous signal.

Adapted from: Lux A, Morita S, Abe J, Ito K, 2005.An improved method for clearing and staining free-hand sections and whole-mount samples. Ann Bot (Lond) 96:989–996.