

Propidium Iodide (PI) Staining

1. Grow seedlings in ½ MS plates vertically for 5 days.
2. Incubate in the dark for 10 min in 15 µM (10 µg/mL in distilled water) propidium iodide (Invitrogen).
3. Rinse in water twice.

Remarks:

- Use enough water when mounting samples. Be careful of not to let the samples dry out.
- Do not squeeze roots; use featherweight forceps.
- Do not touch the root when holding the seedlings, hold them from the cotyledons.
- Avoid pressing the cover slip, otherwise the PI may penetrate the root and stain the xylem.
- Do not analyze seedlings that have been in PI for longer than 30 min.
- Do not use half MS for incubation with PI.

Counting:

Excitation and Emission: 488 nm, 500–550 nm

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.

Adapted from: Alassimone J, Naseer S, Geldner N, 2010. A developmental framework for endodermal differentiation and polarity. *Proc Natl Acad Sci USA* 107:5214–5219.