

Propidium Iodide (PI) Staining

1. Grow seedlings in $\frac{1}{2}$ MS-agar plates vertically for 5 days.
2. Incubate in the dark for 10 min in 15 μM (10 $\mu\text{g/mL}$) propidium iodide (PI) (Invitrogen).
3. Rinse twice in water.

Remarks:

- Use enough water when mounting the samples. Be carefull of not letting 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.

Counting:

Excitation and Emission wavelengths: 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.