

## **Propidium Iodide (PI) Staining**

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

### **Remarks:**

- Use enough water when mounting the samples. Be careful 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.