

## **Arabidopsis Seed Sterilization Protocol (September, 2005)**

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### **Notes**

Do this >2-3 days in advance for AR4 (Col), AR6 (DijonG), AR10 (Ler), AR13 (No-O), and recommended 7D in advance for AR7 (est) to allow for a 4C vernalization treatment to promote more uniform germination. Other ecotypes/genotypes may vary.

Volumes are not absolute – only approximate is fine. Can scale down.

The bleach/Silwet solution can be stored for several weeks at 4C (not at room temp)

### **Protocol**

1. Count seed and place in 15ml blue-capped tubes.
2. The remaining steps should be performed in a sterile tissue culture hood.
3. Add 10ml of 15% bleach (in sterile ddH<sub>2</sub>O) with Silwet (50ul per 500 ml).
4. Shake vigorously until seeds are no longer clumping (alternatively can vortex for 30s). Leave tubes sitting on side for 15-20min, and rotate occasionally.
5. Let seeds settle to bottom, then pour off bleach solution.
6. Add 10ml sterile ddH<sub>2</sub>O to rinse out bleach. Let seeds settle. Pour off ddH<sub>2</sub>O.
7. Replace with 10ml 70% ethanol. Shake tubes continuously for 30-45sec. Let seeds settle to bottom. Pour off ethanol.
8. Replace with 10ml sterile ddH<sub>2</sub>O. Let seeds settle. Pour off ddH<sub>2</sub>O. Repeat 4X.
9. Leave in 10 mL sterile ddH<sub>2</sub>O. Store at 4C for >2days (see note above).