

## Seed surface sterilization for *Artemisia tridentata* ssp. *tridentata*

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The purpose of this protocol is to surface sterilize seeds, thereby reducing chance of contamination when starting plants in culture. Surface sterilization needs to occur in a laminar flow hood. Before beginning, have ~500 mL of DI water, 200mL beaker and 50 mL beaker autoclaved. Seeds must be placed in culture immediately after sterilization.

### **Equipment:**

1 L flask or beaker (for autoclaved DI water)  
200 mL beaker (autoclaved)  
50 mL beaker (autoclaved)  
Dissecting scope  
Glass petri dish  
160  $\mu$ m cheese cloth  
Cut falcon tube with lid hollowed out (see attached picture)  
Long, thin forceps  
Support stand  
Clamp (attaches to support stand)  
Timer (phone will do)  
Laminar Flow Hood

### **Reagents:**

0.1% Triton X-100 (CAS 9005-64-5)  
10% Bleach solution  
70% Ethanol (spray bottle)  
95% Ethanol (falcon tube or coplin jar)

### **Step 1 (count seeds) ~1 hr:**

- Pour small amount of seeds into plastic weigh boat or glass dish.
- Using dissecting scope and forceps, count and place seeds into another glass dish.

**NOTE:** For *Artemisia tridentata* ssp. *tridentata* there is ~50% germination rate of seeds.

### **Step 2 (wash seeds) ~1.5 hrs:**

- Place 160 $\mu$ m cheese cloth into lid of falcon tube (see attached picture)
- Place seeds into falcon tube, onto cheese cloth.
- Secure falcon tube to support stand using a clamp and place under running DI water.
- Make sure water is running over cheese cloth, “washing” the seeds.

**NOTE:** Washing seeds for 3 hrs has been done in the past, 1.5 hrs seems to be sufficient.

### **Step 3 (while seeds are washing):**

- If using a bead sterilizer, turn on (takes about 45 min to warm up)
- Take doors off Laminar Flow hood, turn on lights, fan, and power outlet

### **Step 4 (Aseptic techniques for laminar flow hood):**

- Bring all items that need to be placed into the hood near the hood.
- Spray 70% ethanol all over the inside of the hood, wipe down.
  - Wipe all flasks and beakers that go into the hood with 70% ethanol.
  - Spray gloved hands with ethanol.

- Place forceps in falcon tube containing 95% ethanol and let sit for a minute.
- Wipe bottom and sides of 200mL beaker with 70% ethanol, bring into hood and add ~100 mL bleach/TritonX solution.
- Bring in washed seeds (keep in falcon tube)
- Place forceps in bead sterilizer for ~30 seconds, dip again in 90% ethanol tube to cool.
- Spray gloved hands with 70% ethanol.

**Step 5 (surface sterilize seeds) 10 min:**

- Keeping seeds in the falcon tube, place entire device into 200 mL beaker with bleach/TritonX solution.
  - You can leave the forceps in the falcon tube to weigh down the device.
  - Set timer for 10 minutes.
  - Stir once in a while.

**Step 6 (rinse seeds) ~20 min:**

- Place falcon tube with seeds into 50 mL beaker, pour enough autoclaved water over seeds to thoroughly cover seeds. Rinse for 5 minutes. Remove falcon tube (using forceps) and pour off rinse water into 200 mL beaker.
  - Repeat this step until seeds have been rinsed 4 times for 5 minutes each.
- Seeds are now ready to be placed in culture

Figure 1: Rinsing seeds in falcon tube.







