

Seed Sterilization (*M. truncatula* Acid scarification)

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1. Count the number of seeds required and place them in a falcon tube 50 mL. (Approximately 0.4 g = 100 seeds)
2. Cover them with HPLC grade sulphuric acid. Allow 10-15 min or till dark brown flecks appear on a majority of the seeds. Start looking for flecks at 8 minutes.
3. Carefully siphon off the acid and discard into a large volume of water. Wash seeds with autoclaved dd-water 5 times or more. The first time discard water asap because it generates heat. Neutralize the acidified water with commercial baking soda. Wash seeds very gently.
4. Add 10% bleach (Chlorox household bleach) to just cover the seeds into each tube for 2 minutes exactly.

Remaining steps are in laminar clean bench.

5. With autoclaved tips remove the bleach into a disposal unit.
6. Wash the seeds repeatedly by adding sterilized water and shaking the capped falcon tube by hand.
7. Leave seeds in water with no bleach for 1-4 hours or till they imbibe water and swell (Colour changes to yellow)
8. Place each seed separately on DWA in deep dish petri plate and store at 4 degree for 3 days wrapped in Aluminium foil and then germinate by placing the inverted plate in the growth room overnight while still wrapped in opaque aluminium foil.