Standard Operating Procedure
for
Hazardous Chemicals

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Building and rooms: Keating Building, Lab 302

Procedure	**Arabidopsis Seed Sterilization and Plating**
Chemicals	MS (Murashige and Skoog) Basal Salt, Vitamin solution, Gellan Gum, Triton X-100, Kanamycin, Timentin, Bleach, Ethanol.
Specific Hazards (refer to MSDS for more detailed information)	Bleach: Corrosive. Ethanol: Flammable.
Personal Protective Equipment	Lab gloves (nitrile or latex), lab coat
Engineering/Ventilation Controls	Emergency shower and eyewash accessible.
Special Handling Procedures and Storage Requirements	MS salt, Vitamin: @ 4°C. Kanamycin, Timentin: @ -20°C. Bleach: stored under the sink.
Spill and Accident Procedures (for hazardous chemicals only)	*Skin exposure*: Wash skin with water for 15-20 minutes. If irritation develops, call a physician. *Eye exposure*: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses, after first 5 minutes. Continue rinsing eye. Call a physician. *Spill*: Containerize liquid and use absorbents on residual liquid; dispose appropriately. Wash area and let dry. For spills of multiple products, responders should evaluate the MSDS's of the products for incompatibility with sodium hypochlorite. Breathing protection should be worn in enclosed, and/or poorly ventilated areas until hazard assessment is complete.
Waste Disposal	Bleach < 10% can be drained into sink with large quantity of water.
Special Approval	No special authorization needed after SOP training & reading MSDSs.
Prepared By and Date	Christopher Frost, 06/01/2015, revised 1/5/2021
Reviewed By and Date	Christopher Frost, 1/5/2021

SOP: Arabidopsis Seed Sterilization and Plating

General Information: 100 ul dry seed volume ≈ 2500 seeds

Petri Dish	Size (mm)	1L media makes:	Each plate holds:	Per 100ul dry seed:
Normal	60 x 15	40 dishes	~1250 seeds	2 plates
Large	100 x 15	20 dishes	~2500 seeds	1 plate

Supplies:

Item	**Supplier**	**Part Number**
Murashige and Skoog (MS) Basal Salt Mixture	Sigma	P8169-100G
Phytagel (Gellan Gum)	Sigma	P8169-100G
Agar (Alternate to Phytagel)	VWR	J637-500G
Petri Dishes (60 x 15)	VWR	25384-092
Petri Dishes (100 x 15)	VWR	25384-342

A. Media Preparation for Arabidopsis Seed Germination:

	500 ml	1L
½ MS Salts (no sucrose, no vitamins)	1 g	2g
Adjust pH to 5.7		
Gellan Gum	1.5g	3g
Autoclave for 25 min/500ml or 40 min/1L& cool to 60^{o}C		
Optional: 50 mg/L Kan [stock = 100 mg/ml], or 5 mg/L Hyg [stock = 100 mg/ml], or other selecting agent	250 ul 25 ul	500 ul 50 ul
Optional: 300 mg/L Timentin [stock = 200 mg/ml]	705 ul	1.5 ml
Pour to plates in sterile hood & allow to solidify		

Also autoclave 0.1% gellan gum in 5-10ml ddH_2O for use in Step B-8 below.

B. Arabidopsis Seed Sterilization and Plating:

If this is a transformation screening, screen > 2,500 seeds (1% transformation efficiency = 25 seeds)

1. Aliquot 100 ul of seeds into a 1.5 ml tube
2. Add 1 ml 70% EtOH, shake or vortex for 5 min (making sure seeds are dispersing freely, not in clumps)
3. Spin briefly, remove and discard supernatant
4. Add 1ml 50% Bleach (with 0.1% or 1ul of Triton X-100), shake for 30 min
5. Spin briefly, remove and discard supernatant
6. Wash with 1ml sterilized water 3 times, for 5 min each, by shaking or vortexing
7. Spin briefly to pellet the seeds, remove and discard supernatant
8. Add 1 ml of sterile water (or autoclaved 0.1% gellan gum) to resuspend seeds
9. Use 1ml pipet tip to dispense the seeds (or seed/gellan slurry) drop by drop onto the plate
10. If necessary, add sterile water to splash seed clumps in order to more evenly distribute the seeds. Remove excess water with a pipette, dry the plate for ~10min.
11. Store @ 4^{o}C for 2 days
12. Place the plates in the growth area under 16h/8h light.

Notes:

- For T2 seeds, 70% EtOH for 5 min and 20% bleach for 10 min usually work well.
- Seeds will germinate in one day @ RT, with an 85-90% germinate rate.
- It may take 2 weeks or longer to kill non-transformed seeds that germinated.
- You may need to adjust the plating density of seeds for specific experiments
- If plating seed one-by-one, 1 small petri dish can plate ~ 60 seeds.