LSOP Title	Preparation of auxin in lanolin for plant treatment
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Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste* *OGTR*
Risk Assessments	
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1.0 Scope

This procedure covers the preparation of auxin (IAA and NAA) in lanolin and extends to setting up lanolin syringes for treatment and making stock solutions of NAA and IAA.

This LSOP does not cover the preparation of other hormones in lanolin.

2.0 Definitions

IAA – Indole-3-acetic acid – endogenous auxin

NAA – 1-Naphthaleneacetic acid – Synthetic auxin

EtOH - Ethanol

3.0 Materials and Equipment

1. Lanolin (stored in glass beaker and jar on chemical shelf above balance)
2. IAA (stored in -20°C freezer on shelf with other hormones)
3. NAA (stored in -20°C freezer on shelf with other hormones)

4. Ethanol (100% EtOH; stored in flammables cupboard)
5. 10 mL syringes (no needle) (located in shelves with sliding glass door, to the left of the fume hood)
6. Blu-tak (located in cupboard on shelf above eppendorf tubes etc.)
7. 1 mL syringes (no needle) (located in shelves with sliding glass door, to the left of the fume hood)

8. Sharpie

4.0 Prescribed Actions

N.B. 1mL lanolin is plenty to treat 50 plants

1. Make IAA + NAA stock solutions for 1000 ppm IAA + 100 ppm NAA (see appendix)
   N.B. we include a spike of NAA in the mixture, allowing for hormone quantification experiments to easily tell if contamination from lab surfaces has occurred.

Chemical	Quantity	Final concentration
NAA	0.01 g / 10 mg	10 $mg.mL^{-1}$
IAA	0.1 g / 100 mg	100 $mg.mL^{-1}$
EtOH	1 mL/ 1000 µL	

2. Melt lanolin until liquid in glass beaker using microwave. If lanolin stock is depleted in beaker, add further lanolin from the jar, using a big spatula (very messy).

3. Decant required volume of liquid lanolin into labelled 10mL syringes with blutak plug at end and mix required volume of EtOH control or IAA/NAA mix, one at a time, as outlined in table below. Mix well with a spatula or long pipette tip, then replace plunger into syringe carefully without ejecting lanolin mixture. Lanolin may require re-melting in microwave between preparation of control and treatment.

Treatment	Control	1000 ppm IAA + 100 ppm NAA (1 $mg.mL^{-1}$ IAA + 0.1 $mg.mL^{-1}$ NAA)
100 $mg.mL^{-1}$ IAA 10 $mg.mL^{-1}$ NAA in EtOH solution	-	50 µL
EtOH	50 µL	-
lanolin	4.95 mL	4.95 mL

4. Wrap syringes in alfoil (auxin oxidises in light) and store at 4°C until ready for use.

5. On day of experiment, take lanolin/auxin stocks wrapped in alfoil on ice to glasshouse/growth room. Take additional 1 mL syringes and blutak (and sharpie for labelling treatment lanolin/auxin solution transferred) to perform treatments. Transfer lanolin mix from 10 mL syringe to 1 mL syringe by dispensing into the top of the 1mL syringe (with 1mL plunger removed). Carefully replace plunger into 1mL syringe, ready for use.

5.0 Appendix

Making NAA and IAA stock solutions:

1. Weigh 10 mg NAA (0.01 g 1-Naphthaleneacetic acid) into a 2 mL Eppendorf tube
2. Dissolve NAA in 100 µL EtOH to give 1 $mg.mL^{-1}$ NAA solution
3. Weigh 100 mg IAA (0.1 g indole-3-acetic acid) into a separate 2 mL Eppendorf tube
4. Add 100 µL 1 $mg.mL^{-1}$ NAA solution to IAA
5. Add a further 900 µL EtOH to NAA+IAA (from step 4) to dissolve IAA, to finally give a 100 $mg.mL^{-1}$ IAA 10 $mg.mL^{-1}$ NAA solution.

N.B Be very careful to not spill or contaminate lab or balance surfaces with auxin. Before starting, put a sheet of alfoil on your working surface and on the balance to catch any spills. Change gloves regularly. Be sure to wash spatula extremely well with decon and lots of water before putting into dishwasher.