

LSOP Title	How to make GR24 treatment solutions for direct pea bud applications
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## 1.0 Scope

*This procedure covers the protocol on how to make GR24 treatment solutions for direct pea bud applications which can be used in phenotypic or gene expression studies.*

## 2.0 Definitions



EtOH - Ethanol

PEG - polyethylene glycol

GR24 – synthetic strigolactone analog

## 3.0 Materials and Equipment

1. Pipette and Pipette Tips
2. Tubes
3. PEG1450
4. GR24
5. EtOH
6. Acetone



## 7. Plants

### 4.0 Prescribed Actions

**LABORATORY STANDARD OPERATING PROCEDURE (LSOP)**

ARC COE for Plant Success in Nature and Agriculture: *How to make GR24 treatment solutions for direct pea bud applications*



1. If using bud treatments for phenotypic experiments, make solution to give the following final concentrations: 50% EtOH, 2% PEG 1450, 0.1% acetone

<b>Solution</b>	<b>100% EtOH</b>	<b>MilliQ Water</b>	<b>8% PEG 1450 (in 50% EtOH)</b>	<b>1mM GR24 (in acetone)</b>	<b>Acetone</b>				<b>Total Volume</b>
Control	375 $\mu$ L	374 $\mu$ L	250 $\mu$ L	-	1 $\mu$ L				1000 $\mu$ L
1 $\mu$ M GR24	375 $\mu$ L	374 $\mu$ L	250 $\mu$ L	1 $\mu$ L	-				1000 $\mu$ L

### Working solution preparation



1. Have ready – 8% PEG1450 and GR24 stock solutions (see appendix) plus 100% EtOH (analytical grade), MilliQ water, acetone.

2. Prepare bud treatment solutions on ice, preferably on the day of the experiment.



*NB: If making solutions in advance (e.g. day or morning before), store treatments solutions in freezer at -20°C (50% EtOH will not freeze).*



3. Take to glasshouse on ice

4. Make enough solution to treat all buds, plus some spare.

*NB: Usually, 10  $\mu$ L solution is applied to each bud.*

## 5.0 Appendix

Making Stock Solutions:

	8% PEG 1450 stock:	Dissolve 4 g PEG 1450 in 50 mL 50% EtOH (made with MilliQ water)
	1mM GR24:	(in acetone) dilute a stock solution down to 1mM