LSOP Title	Tre6P Sugar Extraction
LSOP No.	LSOP34
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *OGTR*
Risk Assessments	
Approved by	*Franziska Fichtner*
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1.0 Scope

To outline the procedures for Chloroform/Methanol extractions of all water-soluble metabolites (including trehalose 6-phosphate)

2.0 Definitions

Eppendorf tube –

Tre6P – Trehalose-6-Phosphate

3.0 Materials and Equipment

1. Speed vac
2. Vortex
3. Eppendorf tubes
4. Micro pestle
5. Ice
6. Liquid nitrogen
7. Ground plant material

8. Weigh balance

9. Chloroform/Methanol
10. Pipette and Pipette Tips
11. Ice cold water

4.0 Prescribed Actions

Chloroform/Methanol extraction

1. weigh ~18 mg (15 to 20 mg range) ground plant material in 2 ml safe-lock Eppendorf tubes (we can also use single pea buds, then the elution volume at the end changes)

2. Add 350 µl Chloroform/Methanol (3/7; icecold, store at -20°C) into the tube which still is placed in liquid nitrogen and mix well.
3. After having done 10 samples vortex all and put into -20°C. Continue with the rest of the samples.
4. After 2 h in -20°C, place the samples on ice again and add 350 µl of ice-cold water, vortex and centrifuge for 10 min at 4°C.
5. Transfer the upper aqueous phase into a new tube (1 ml screw cap)

6. Re-extract the lower Chloroform phase with 300 µl of ice-cold water.
7. Combine the water phases and evaporate to dryness in the speed vac.
8. Dissolve samples in 400 µl water (this depends on how much material we have and can change, for single buds use less)

For Tre6P measurements on LC-MS/MS

- filter through a MultiScreen PCR-96 Filter Plate membranes (Merck Millipore, www.merckmillipore.com) to remove high molecular weight contaminants.