



## APPLICATION NOTE

### Perchloric Acid Extraction of Leaves for Measurement of Starch and Soluble Metabolites

With kind permission of Dr. Marilyn Pike from The John Innes Centre, Department of Metabolic Biology, Norwich, UK

#### Experiment

The harvested plant material (up to about 300 mg) is pre-weighed into 5ml polycarbonate vials (SPEX SamplePrep cat no. 2240- PC) suitable for use in Geno/Grinder® instrument. The tubes are then capped and placed immediately into liquid nitrogen (the time from harvest to freezing should be as short as possible) NB: Plant material can be harvested and frozen in any cryo-vial, then transferred to the Geno/Grinder tubes just before grinding if necessary. Then transfer the tubes to dry ice to keep material frozen and add 500 µl ice cold 0.7M perchloric acid to each tube. Allow the acid to freeze. Place one good quality stainless steel ball, 10 mm diameter in 316 grade material, into each vial. Cap the tubes securely and place into the grinder foam holder.

Place and secure holder containing tubes into machine as per Geno/Grinder instructions. Grind samples for 90 sec at 1500 strokes per minute or until sample is smoothly pulverized. Transfer the tubes to ice immediately after grinding. Add an additional 2500 µl ice cold 0.7M perchloric acid to each tube and vortex briefly. Centrifuge for 10 min at 4° C and 14,000 xG.

Remove and neutralise the resulting supernatant to pH 6-7 by addition of 2 M KOH, 0.4 M MES, 0.4 M KCl. After centrifugation to remove the insoluble perchlorate, the neutralised solution can be used for assay of soluble metabolites. All steps should be carried out at 0-4 °C. The pellet contains the starch. After washing with water to remove the perchloric acid, it can be used for assay of starch according to Smith and Zeeman (2006).

#### Reference

Smith AM and Zeeman SC (2006) Quantification of starch in plant tissues. Nature Protocols 1, 1342-1345.

∴ APPLICATION NOTE SP013:  
Grinding/Disruption

∴ APPARATUS:  
**Geno/Grinder®**

∴ APPLICATION:  
Starch and Soluble Metabolite  
Measurement in Leaves



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