Version 2

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Grinding Tissue with the Qiagen Tissuelyzer (Soybean, Cowpea, Tobacco) V.2

Version 1 is forked from Leaf Protein Extraction for Immunoblot (Soybean, Cowpea, Tobacco)

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

Grinding tissue using the Qiagen Tissuelyzer for DNA extraction, RNA extraction, or Immunoblotting.

Note: when using the TissueLyser II it is recommended to use 2 mL centrifuge tubes in conjunction with 4mm beads. 1.5mL tubes are narrow at the end and samples will not grind properly if you use the 4mm beads.

PROTOCOL CITATION

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https://protocols.io/view/grinding-tissue-with-the-qiagen-tissuelyzer-soybea-bt4ynqxw
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FORK NOTE

FORK FROM

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MATERIALS TEXT

- TissueLyser II (QIAGEN; 85300)
- 2 mL centrifuge tubes, Fisher Scientific 14-666-313
- 4mm SPEX^{TM} stainless steel grinding beads (SPEX; 2150)
- 13.4 mm diameter, flash-frozen leaf disks
- Ethanol, CAS 64-17-5
- Beaker, 100 mL, Fisher Scientific 05-404-119
- Liquid Nitrogen Dewar, Thermo-Fisher 4150-2000PK
- Extra-Long Tongs, Nickel Plated Steel, 18 in, Grainger 5ZPT6
- Cryogenic safety gloves, Fisher Scientific 17-355-050
- Forceps, Fisher Scientific 13-812-211
- Dry ice

SAFETY WARNINGS

Cryogens pose a risk of extreme cold, asphyxiation, and projectiles due to pressure buildup. Before handling cryogens, review the UIUC DRS documentation for Biological Sample Storage in Liquid Nitrogen, Cryogens and Dry Ice, and complete the Compressed Gases and Cryogens Safety Training module. Wear appropriate PPE when handling including safety goggles, lab coat, thermal gloves, and closed toe shoes.

BEFORE STARTING

Receive proper training from lab manager. Familiarize yourself with the Qiagen TissueLyser II User Manual.

1 Pre-cool components for tissue lysis.

1.1 Pre-cool and sterilize 4mm SPEX stainless steel grinding beads in 100% ethanol in a beaker at **-80 °C** for **00:30:00** 30m

1.2 Pre-cool the empty TissueLyser inserts for LN2 for **00:30:00** by lowering the inserts into a full liquid nitrogen dewar with the extra-long tongs. 30m

Wear lab coat, goggles, cryogen safe gloves, and closed toe shoes.

***WARNING: The equipment manufacturer, Qiagen, does not support this practice. Manufacturer recommends precooling inserts at **-80 °C** in a ultra low freezer or on ground dry ice.

Manufacturer does not guarantee robustness or safety of equipment below **-80 °C** . ***

Liquid nitrogen cooling (**-196 °C**) maintains highest sample integrity for nucleic acid extraction and analysis but there is a risk of damaging equipment and it will void any manufacturer warranties on equipment.

2 Prepare sample tubes for tissue lysis

2.1 Remove sample tubes from **-80 °C** storage or liquid nitrogen, if grinding immediately after sampling. Place samples in crushed dry ice.

2.2 Using forceps cooled in LN_2, add one sterilized, pre-cooled SPEX bead per sample tube.

2.3 Remove Tissuelyser II cassettes from liquid nitrogen using extra-long tongs. Insert tubes into pre-cooled TissueLyser II cassettes, ensuring a balanced number of samples between cassettes.

3 Grind tissue for **00:01:30** at 20 Hz

1m 30s

Warning: Do not exceed this frequency, higher frequencies increase the number of cases where the steel beads will break the lid of centrifuge tubes resulting in sample loss. If using the SPEX 2150 beads it is necessary to use 2 mL centrifuge tubes to ensure proper grinding, in 1.5 mL tubes the bead will not reach the tapered bottom leaving samples unground

TissueLyser II
Bead Mill
QIAGEN 85300

4 Remove cassettes from TissueLyser II, keeping samples in them, and submerge them in LN_2 using extra long tongs to prevent thawing. When liquid nitrogen has stopped bubbling, cassettes are ready to reinstall on the TissueLyser II.

Hold the cassette containing samples closed with the extra long tongs in the liquid nitrogen until bubbling has stopped or sample tubes may be dislodged from the cassette by the bubbling.

5 Grind tissue for **00:01:30** at 20 Hz

1m 30s

This repeat is to ensure all tissue is correctly grounded. There will be odd instances where leaf tissue has not properly ground for reasons such as samples sticking to the edge of tubes, check for this on removal, it may be necessary to repeat again.

6 Remove tubes with forceps cooled in LN_2. Return to crushed dry ice for further analysis. Alternatively, samples can be stored at **-80 °C** for several months before processing further.