Version 2

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Leaf Protein Extraction for Immunoblot (Soybean, Cowpea, Tobacco) V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bqnmmvc6

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ABSTRACT

This protein extraction protocol was developed for analysis of protein abundance in leaf tissue by immunoblot. It was optimized for dicot species including Glycine max.

Quantities - 450 µL protein extraction buffer (PEB) per three 13.4 mm diameter leaf disks (size #7 Humboldt Cork Borer)

Note from Agrisera: Keeping sample volumes in a range of 0.2-0.5 mL has been found to contribute to better extraction results, an upscale in volume is not recommended, if no cork borer available the suggestion is 500 µL PEB per 100 mg of plant tissue.
- Final volume ~400 µL
- Expected yield: 1.5 - 6 µg / µL
- Total yield: 6 - 24 mg protein
- Amount of total soluble protein to load per lane 30 µg

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.

DOI

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KEYWORDS

Glycine max, soybean, protein extraction

IMAGE ATTRIBUTION

Image reproduced from the QIAGEN website https://www.qiagen.com/us/products/human-id-and-forensics/automation/tissuelyser-ii/#orderinginformation

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MATERIALS TEXT

- TissueLyser II (QIAGEN; 85300)
- 2 mL centrifuge tubes
- 4mm SPEX^{TM} stainless steel grinding beads (SPEX; 2150)
- Humboldt brass cork borer set (07-865-10B; Fisher Scientific)
- 13.4 mm diameter, flash-frozen leaf disks
- 4x Protein Extraction Buffer (PEB) (8 % SDS (w/v); 40 % glycerol (v/v); 0.25 M Tris HCl (pH 6.8))

(from Abcam) SDS grade is important for high-quality protein separation: a protein stained background along individual gel tracts with indistinct or slightly distinct protein bands are indicative of old or poor quality SDS. This buffer is essentially the same as Laemmli buffer so proteins can be directly loaded onto PAGE gels.

SAFETY WARNINGS

Perform steps with protein extraction buffer in a fumehood

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BEFORE STARTING

Prepare a working solution of 1x Protein Extraction Buffer

- 1x PEB (10 mL) (**2.5 mL** 4x PEB; **250 µl** 2-mercaptoethanol; **100 µl** protease inhibitor cocktail; **7.3 mL** dH_2O)

Buffer Preparation 20m

1 Incubate 4x PEB (if stored at 4 ^{o}C) at **50 °C** for **00:20:00** to re-suspended precipitated SDS in buffer. 20m

2 Make **500 µl** 1x PEB per sample by diluting 4x stock with dH_2O.

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It is advised to make more 1x PEB than necessary to avoid running out.

Tissue Lysis 1h 3m

3 Pre-cool components for tissue lysis

3.1 Pre-cool 4mm SPEX stainless steel grinding beads at **-80 °C** for **00:30:00** 30m

3.2 Pre-cool the TissueLyser inserts for LN_2 for **00:30:00** 30m

4 Prepare sample tubes for tissue lysis

4.1 Using forceps cooled in LN_2, add one SPEX bead per sample tube.

4.2 Insert tubes into pre-cooled TissueLyser II cassettes, ensuring a balanced number of samples between cassettes.

5 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

6 Remove cassettes from TissueLyser II and submerge them in LN_2 to prevent thawing

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7 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.

8 Remove tubes with forceps cooled in LN_2. Samples can be stored at **-80 °C** for several months before processing further.

It is advised not to proceed directly from samples in LN_2 to the next step as they take longer to thaw and resuspend in extraction buffer. A period of 30 mins on dry ice can be beneficial.

Dissolve ground powder 1h 3m

9 Add **450 µl** 1x PEB to ground powder (3x 1cm leaf disk).

10

1m

Vortex immediately (maximum speed) to mix. Approximately **00:01:00**

No lumps should be visible at this stage if the sample is ground well.

11 Heat samples at **95 °C** for **00:05:00** to denature proteins and inactivate proteases.

5m

Prolonged heating can cause cleavage of peptide bonds leading to artifacts. Do not delay heating after sample buffer addition as not all proteases are denatured by SDS and partially denatured peptides are sensitive to protease degradation.

12 Vortex heated samples to shear nucleic acids (**00:00:05** ; max speed)

5s

13 Spin samples for **00:03:00** at **10000 x g, Room temperature** to pellet insoluble material

3m

the pellet should be white/light-grey an intense green color of the pellet can indicate that disruption was not

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optimal and extraction conditions need to be adjusted (e.g. improved grinding, or adjusting buffer volume)

14 Transfer supernatant to a new 1.5 mL centrifuge tube and transfer to ice or store at **-20 °C** for up to a month

Be careful not carry over debris

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