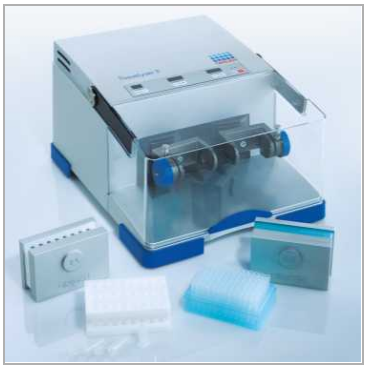

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Protocol status: Working
We use this protocol and it's working

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Leaf Protein Extraction for Immunoblot (Soybean, Cowpea, Tobacco)

Forked from Leaf Protein Extraction for Immunoblot (Soybean, Cowpea, Tobacco)

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Realizing Increased Photosynthetic Efficiency (RIPE)

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.

IMAGE ATTRIBUTION

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

MATERIALS

- 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))

Note

(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

BEFORE START INSTRUCTIONS

Collect tissue immediately into liquid nitrogen and store at -80C. Grind per protocol "Grinding Tissue with the Qiagen Tissuelyzer".

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.

Note

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

Dissolve ground powder

1h 3m

3 Add 450 µL 1x PEB to ground powder (3x 1cm leaf disk).

4 Vortex immediately (maximum speed) to mix. Approximately 00:01:00 1m

Note

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

5 Heat samples at 95 °C for 00:05:00 to denature proteins and inactivate proteases. 5m

Note

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.

6 Vortex heated samples to shear nucleic acids (00:00:05 ; max speed) 5s

7 Spin samples for 00:03:00 at 10000 x g, Room temperature to pellet insoluble material 3m

Note

the pellet should be white/light-grey an intense green color of the pellet can indicate that disruption was not optimal and extraction conditions need to be adjusted (e.g. improved grinding, or adjusting buffer volume)

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

Note

Be careful not carry over debris