FOCUS™ Plant Proteome

(Cat. # 786-259)

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INTRODUCTION

FOCUS™ Plant Proteome is specifically designed for plant research and supplied with plant specific reagents; including reagents for removal of plant pigments and other natural products that may interfere with protein analysis. This kit extracts and solubilizes nearly all of the proteins from plants, including membrane as well as soluble proteins. It is supplied with a proprietary strong chaotropic extraction buffer to solubilize even the most difficult proteins. A complete separate Perfect-FOCUS™ (Cat # 786-124) kit is supplied for preparing sample for 2D gels.

ITEM(S) SUPPLIED (Cat. # 786-259)

Description	Size
FOCUS™ Protein Solubilization Buffer [FPS Buffer]	25 g
DILUENT- III	30 ml
UPPA™ -I	15ml
UPPA™ -II	15ml
FOCUS™-Wash	2ml
OrgoSol Buffer™	50ml
SEED™	300µl
PerfectFOCUS™ Buffer-I	2ml
PerfectFOCUS™ Buffer-II	0.5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Store the kit components as individually marked upon arrival.

ADDITIONAL ITEMS REQUIRED

Sonicator, Centrifuge, Centrifuge tubes, Glass beads, Reducing agent, Carrier ampholytes, and Protease inhibitor cocktail.

PREPARATION BEFORE USE

The kit is supplied with FPS Buffer and DILUENT-III**.** Allow the FPS Buffer to warm to room temperature before opening the bottle. Read the instructions on the bottles carefully before use. Just before use, hydrate an appropriate amount of the FPS Buffer with the DILUENT-III. Add needed agents such as reducing agent, and if necessary an appropriate amount of protease inhibitor cocktail (e.g. FOCUS-Protease Arrest™, Cat. # 786-108F).

PROTOCOL

1. Weigh appropriate amount of plant tissue and cut in to small pieces. Transfer the tissue to a centrifuge tube and add 2-3ml freshly prepared FPS Buffer per gram tissue. Sonicate the suspension with an ultrasonic probe to break down the tissues. Sonication should be performed in cold (ice cold bath) and during sonication care must be taken to prevent heating. Sonication should be performed with bursts of 30-40 seconds. Chill the suspension between ultra-sonic bursts.
2. It may take 10 - 30 minutes depending on the sample size and the sonication strength. Adding 2-3ml glass beads (1mm diameter) per gram tissue will facilitate disrupting of the tissues.
3. Alternatively, grind the tissue in liquid nitrogen first. Then add fresh FPS Buffer and mix well. Other types of mechanical grinders may also be used.
4. Centrifuge at 20,000 x g for 30 minutes at 20°C and collect clear lysate.
5. Some plant debris can't form tight pellet after centrifugation, you may need to filter the lysate instead of centrifugation or filter the supernatant after centrifugation.
6. Suspend the residual cell debris in 1/4 the volume of FPS Buffer used in the previous Step-1. Sonicate the suspension once briefly. Repeat step 2. Collect the extract and pool with the first extract supernatant. Store total protein extract at -70°C until used.
7. Determine the protein concentration. We recommend our Non-Interfering Protein Assay kit, Cat. #786-105.
8. Take appropriate amount of lysate and use Perfect -FOCUS kit to clean the proteins (See Processing Fractions for IEF/2D Analysis).
9. Suspend the protein pellet in freshly prepared FPS Buffer with an ampholyte and reducing agent for running IEF/2D gels if necessary.

NOTE: *Proteins solubilized in FPS Buffer that contain CHAPS may not be suitable for running SDS-PAGE. If you want to run SDS-PAGE with the plant sample, use the protein pellet obtained in Step-5 above.*

PROCESSING FRACTIONS FOR IEF/2D ANALYSIS

For IEF/2D gel analysis, use an appropriate amount of the membrane Protein Fraction, process only as much protein as you need (i.e. 50-200µg protein /run).

Important Notes

- Perform the entire procedure at 4-5°C (ice bucket) unless specified otherwise. Various incubation conditions must be strictly followed. Use 1.5ml microfuge tubes for processing protein samples. 0.5ml microfuge tubes are not recommended.
- Always position the microfuge-tubes in the centrifuge in the same orientation, i.e. cap-hinge facing outward. This will allow the pellet to remain glued to the same side of the tube during centrifugation and washing steps and minimize the loss of the protein pellets.
- Chill OrgoSol Buffer at –20°C for ~1hr or more before use

Protocol

1. Transfer 1-100µl protein solution (containing 1-100µg protein per sample) into a 1.5ml microfuge tube.
2. Add 300µl UPPA-I and mix well. Incubate at 4-5°C (ice-bucket) for 15 minutes.
3. Add 300µl UPPA-II in to the mixture of protein and UPPA-I, then vortex the tube.
   NOTE*: For larger sample size, use 3 volumes each of UPPA-I and UPPA-II for each volume of sample. See Appendix: Processing Large Samples.*
4. Centrifuge the tube at 15,000x g for 5 minutes to form a tight protein pellet.
5. As soon as the centrifuge stops, remove the tube from the centrifuge.
   NOTE*: Pellets should not be allowed to diffuse after centrifugation is complete.*
6. Carefully, without disturbing the pellet, use a pipette tip to remove & discard the entire supernatant.
7. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing out-ward. Centrifuge the tube again for 30 seconds. Use a pipette tip to remove the remaining supernatant.
8. Add 40µl of FOCUS-Wash on top of the pellet. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing out-ward.
   NOTE: *For larger sample size, add Wash 3-4 x times the size of the pellet.*
9. Centrifuge the tube again for 5 minutes. Use a pipette tip to remove and discard the Wash.
10. Add 25µl of pure water on top of the pellet.
    NOTE: *For large sample size, add water just enough to cover the pellet, i.e. a volume equal to the size of the pellet.*
11. Vortex the tube.
    NOTE: *Pellets do not dissolve in water*.
12. Add 1ml OrgoSol Buffer, pre-chilled at –20°C, and 5µl SEED.
    NOTE: *For large samples size, for each 0.1-0.3ml protein solution add 1ml OrgoSol Buffer. In addition, OrgoSol Buffer must be at least 10 fold in excess of the water added in Step 10.*

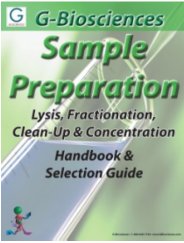
13. Vortex to suspend the pellet. It is important that the pellet is fully suspended in OrgoSol Buffer.
    NOTE: *Pellets do not dissolve in OrgoSol Buffer.*
14. Incubate the tube at –20°C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.
15. Centrifuge at 15,000xg for 5 minutes to form a tight pellet.
16. Remove and discard the supernatant. You will notice a white pellet in the tube. Air-dry the pellet. On drying, the white pellet will turn translucent.
    NOTE*: Do not over dry the pellets - parched dry pellets may be difficult to dissolve*.
17. Add an appropriate volume of hydrated FPS Buffer to suspend the pellet. Vortex the tube for 30 seconds. Incubate and vortex periodically until pellet is dissolved. Centrifuge and collect a clear protein solution and load on IEF gel.

Alternative Protein Solubilization Protocol

1. Add 5-40µl PerfectFOCUS Buffer-I to the pellet and vortex.
2. Incubate at room temperature for 5 minutes.
3. Add PerfectFOCUS Buffer-II and vortex for 30 seconds For each 5µl PerfectFOCUS Buffer-I used, add 1µl of PerfectFOCUS Buffer-II.
4. Vortex and incubate at room temperature for 5 minutes to completely dissolve the protein pellet. The protein solution at this stage contains 60mM Tris, pH 7-7.5.

RELATED PRODUCTS

Download our Sample Preparation Handbook



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/

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