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A Geno Technology, Inc. (USA) brand name

FOCUS™ Soluble & Insoluble Protein Extraction

(Cat. #786-247)

INTRODUCTION

A complete kit for the selective preparation of soluble (hydrophilic) and insoluble (hydrophobic) proteins from mammalian tissues and cells, plants, yeast, bacteria, and other biological samples. The kit comes with reagents necessary for fractionation of soluble and insoluble fractions, including a strong chaotropic extraction buffer to solubilize difficult proteins.

The FOCUS™ Soluble & Insoluble kit is supplied with a specific clean-up kit for the preparation of each fraction for isoelectric focusing and 2D electrophoresis for improved spot resolution.

FOCUS™ Soluble & Insoluble kit is designed for 50 preps, where one prep is:

- 100mg mammalian tissue
- 50µl wet animal cell pellet
- 50µl wet yeast pellet
- 50µl wet bacteria pellet
- 250mg plant tissue

ITEM(S) SUPPLIED (Cat. # 786-247)

Description	**Size**
Soluble Protein Extraction Buffer [SPE Buffer]	50ml
FOCUS™ Protein Solubilization Buffer [FPS Buffer]	25g (For 50ml)
FOCUS™ Extraction Buffer DILUENT-III	30ml
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 Temp. Upon arrival, store *Perfect*-FOCUS™ at room temperature and rest of the kit components in cold at ~ 5-10°C.

ADDITIONAL ITEM(S) REQUIRED

Centrifuge, centrifuge tubes, reducing agent, alkylation agents, carrier ampholytes and protease inhibitor cocktail.

PREPARATION BEFORE USE

1. The kit is supplied with a FPS Buffer and DILUENT- III. Allow the FPS Buffer to warm to room temperature before opening the bottle. Read the instructions on the bottle labels carefully before use. Just before use, hydrate an appropriate amount of the FPS Buffer with DILUENT-III. Add needed agents such as reducing agent, carrier ampholyte, and if necessary an appropriate protease cocktail.
2. **Protease Inhibition:** If the inhibition of protease activity is required; add a cocktail of protease inhibitors in MPE Buffer-I to prevent protease activities during extraction procedure.

PROTOCOL

1. For each 100 mg of tissue, use approximately 0.4-0.5ml SPE Buffer.
For each 50µl of wet animal cell pellet, use approximately 0.4-0.5ml SPE Buffer.
For each 50µl wet yeast pellet, use 0.4ml SPE Buffer**.**
For each 50µl wet *E. coli* pellet, use 0.4ml SPE Buffer.
For each 1gm plant tissue, use 2ml SPE Buffer-I.
NOTE: *The sample to buffer volume ratio specified above is only a guide and may be adjusted depending on the scale of preparation.*

2. Sonicate the suspension with an ultrasonic probe to break the cells and break down the genomic DNA. 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 20-30 seconds and chill the suspension between ultra-sonic bursts.
NOTE: *Disruption of cells depends upon the nature of cells. E. coli cells require longer sonication than animal cells and tissues. Yeast cells require even more vigorous sonication. Addition of glass beads in the yeast cell suspension greatly facilitates disruption of yeast cells.*
3. Centrifuge the homogenate at 20,000xg for 30 minutes at 4-5°C to pellet the debris.
4. Use a pipette to remove the clear supernatant without disturbing the pellet.
5. Suspend the pellet in 1/4 the volume of SPE Buffer used in Step 1.
6. Repeat Steps 2-4. Collect the clear supernatant and pool with the first supernatant.
7. Resuspend the pellet in 0.5ml SPE Buffer, vortex for 60 seconds, and centrifuge at 20,000xg for 15 minutes at 4-5°C. Remove and discard the wash.
OPTIONAL: *Wash may be saved or pooled for further analysis.*
8. Mark the fractions as follows:
    - Clear Supernatant: Soluble Protein Fraction [Hydrophilic Fraction]
    - Cellular Debris & Pellet: Insoluble Protein Fraction [Hydrophobic Fraction]
9. Store both fractions at -70°C until used.
10. Determine the protein concentration of the soluble protein fraction. We recommend using Non-Interfering Protein Assay (Cat. # 786-005).

PROCESSING SOLUBLE FRACTIONS FOR IEF/2D ANALYSIS

For IEF/2D gel analysis, use an appropriate amount of the fractions, 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.
    NOTE*: The Soluble Protein Fraction may be directly mixed with FPS Buffer for running IEF/2D analysis. If the Soluble Protein Fraction is sufficiently concentrated, you may mix 1 part Soluble Protein Fraction with >20 parts FPS Buffer without seriously diluting the FPS Buffer.*

PROCESSING INSOLUBLE PROTEIN FRACTION FOR IEF/2D ANALYSIS-

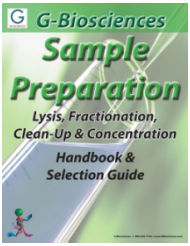
1. Suspend the insoluble protein in 0.3-4ml FPS Buffer. Vortex the suspension 4-5 times, 60 seconds each, to solubilize the insoluble protein.
2. Centrifuge 20,000xg for 30 minutes at 15-20°C and collect the clear supernatant.
3. Resuspend any residual pellet in 1/4 the volume of FPS Buffer used in Step 1. Centrifuge 20,000xg for 30 minutes at 15-20°C and collect and pool the clear supernatant.
4. Determine the protein concentration of the soluble protein fraction. We recommend using Non-Interfering Protein Assay (Cat. # 786-005).
5. Make an appropriate dilution in FPS Buffer before running IEF/2D gels.
   NOTE: *Depending on the source and the nature of the sample, some insoluble materials (debris) may be recovered after the extraction steps. For solubilization of difficult-to-extract proteins, you may try the range of specialized FOCUS-Extraction Buffers we offer. Visit www.GBiosciences.com for more information or contact our Technical Support.*

Cleaning of Protein Extract for 2D Analysis

Depending on the nature of the samples, sometimes it is necessary to clean the protein extracts before running IEF/2D analysis. Follow the procedure outlined for the soluble protein fractions

RELATED PRODUCTS

Download our Sample Preparation Handbook.



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

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