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A Geno Technology, Inc. (USA) brand name

FOCUS™ Membrane Proteins

(Cat. # 786-249)

INTRODUCTION

FOCUS™ Membrane Proteins kit is specifically designed for a simple, rapid and highly reproducible method for preparation of membrane or hydrophobic proteins from biological samples for 2D-gel analysis or other applications. This kit does not require the use of difficult to prepare gradients or the use of expensive ultracentrifuge equipments. This kit consists of reagent solutions consisting of non-ionic detergents, which allows isolation of membrane by temperature dependent phase partition [1-2]. Protein sample is mixed, homogenized or suspended in the membrane extraction buffer. After a brief incubation at 35-37°C, the sample is centrifuged which results in separation of a membrane (hydrophobic) protein rich layer. Proteins anchored to the membrane or proteins containing one or two trans-membrane regions are extracted into the membrane rich protein layer with the efficiency higher than 50%. Lower efficiency may be obtained with more complex membranes. The membrane (hydrophobic) preparation is suitable for most applications including SDS-PAGE, Western blotting, 2D-gel analysis, etc. The kit is suitable for 50-100 preps (depending on sample size).

ITEM(S) SUPPLIED (Cat. # 786-249)

Description	Size
MPE Buffer-I [Membrane Protein Extraction Buffer-I]	50ml
MPE Buffer-II [Membrane Protein Extraction Buffer-II]	50ml
FOCUS™ Protein Solubilization Buffer [FPS Buffer]	25g
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 temperature. Store the kit components as individually marked.

ADDITIONAL ITEMS 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.
3. MPE Buffer-I & MPE Buffer-II- Before use make sure the buffers are chilled, alternatively, place the buffers in ice-bath for 10-15 minutes and invert the bottle 2-3 times to mix the content.

PROTOCOL

1. For each 100mg of animal tissues, use approximately 0.2-0.3ml MPE Buffer-I.
   For each 50µl of wet animal cell pellet, use approximately 0.2-0.3ml MPE Buffer-I.
   For each 50µl wet yeast pellet, use 0.25ml MPE Buffer-I**.**
   For each 50µl wet *E. coli* pellet, use 0.25ml MPE Buffer-I.
   For each 1gm plant tissue, use 1ml MPE 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.
3. 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.
4. Add an equal volume of chilled MPE Buffer-II into the suspension.
5. Vortex the suspension 4-5 times, 60 seconds each. Hold the suspension in ice-cold bath between vortexing. Incubate the suspension in ice-cold bath for 10 minutes.
6. Transfer the suspension to a 35-37°C heating block or incubator. Incubate for 30 minutes. Vortex the suspension periodically, 3-4 times 30-40 seconds each.
7. Centrifuge the tube at 18,000x g for 5 minutes at room temperature.
8. Examine the tube carefully. You will notice two visible phases. Remove the top layer and transfer to a clean tube.

9. Add the same volume of chilled MPE Buffer-II as used in Step-4 into the tube containing the bottom layer.
10. Repeat steps 5-7. Remove the top layer and pool with the top layer collected earlier (Step 8).
11. Collect the bottom. Save and store the inter-phase and the sediment at -70°C until the analysis is complete.
12. Mark the Tubes as follows:
    - **Top Layer:** Hydrophilic Protein Fraction
    - **Bottom Layer**: Hydrophobic Membrane Protein Fraction
13. Determine protein concentration of the membrane protein fraction. We recommend using Non-Interfering Protein Assay (Cat. # 786-005).

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.
    NOTE*: The Membrane Protein Fraction may be directly mixed with hydrated FPS Buffer for running IEF/2D analysis. If the Membrane Protein Fraction is sufficiently concentrated, you may mix 1 part Membrane Protein Fraction with >20 parts hydrated FPS Buffer without seriously diluting the FPS Buffer.*
    NOTE*: Hydrophilic proteins may also be processed for IEF/2D analysis using PrefectFOCUS kit as described above for the membrane protein fraction.*

REFERENCES

1. *Towards the recovery of hydrophobic proteins on two-dimensional electrophoresis gels. Santoni. V., Rabilloud. T., Doumas. P., Rouquie. D., Manbsion. M., Kieffer. S., Garin. J., and Rossignol. M. (1999) Electrophoresis, 20, 705-711.*
2. *Preparation of mammalian plasma membranes by aqueous two-phase partition. Morre, J.D., and Morre, D. M. (1989). BioTechniques 7(9), 946-958*

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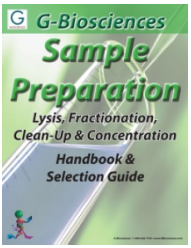

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