



FOCUS™ Membrane Proteins

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).

ITEMS SUPPLIED

Cat # 786-249

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
<i>Perfect-FOCUS™</i> [Cat # 786-124]	1 Kit

STORAGE CONDITION

Shipped at ambient temperature. Upon arrival store *Perfect-FOCUS™* kit at room temperature. Store rest of the kit components as individually marked.

ITEMS NEEDED BUT NOT SUPPLIED

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 (see Related Products for protease inhibitor *Protease Arrest™*).
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 0.05ml of wet animal cell pellet, use approximately 0.2-0.3ml MPE Buffer-I.
Yeast - for 0.05ml wet yeast pellet use 0.25ml MPE Buffer-I.
Bacteria- for 0.05ml wet *E. coli* pellet use 0.25ml MPE Buffer-I.
Plant - use 1ml MPE Buffer-I for each 1gram plant tissue.



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. 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. Add an equal volume of pre-chilled Membrane Protein Extraction Buffer-II (MPE Buffer-II) into the suspension. 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.
4. 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.
5. Centrifuge the tube at 18,000x g for 5 minutes at room temperature.
6. Examine the tube carefully. You will notice two visible phases. Remove the top layer and transfer to a clean tube.
7. Into the tube containing bottom layer, add pre-chilled MPE Buffer-II- the same volume as used in the previous Step-3. Vortex the suspension 3-4 times, 60 seconds each. Repeat Steps 3 - 6. Remove the top layer and pool with the top layer collected earlier (Step 6).
8. Collect the bottom. Save and store the inter-phase and the sediment at -70°C until the analysis is complete.

Mark the Tubes as follows:

Top Layer	Hydrophilic Protein Fraction
Bottom Layer	Hydrophobic Membrane Protein Fraction

Processing "Membrane Protein Fraction" for IEF/2D Analysis - Determine protein concentration of the membrane protein fraction (use Non-Interfering Protein Assay, Cat # 786-005). For IEF/2D gel analysis, use an appropriate amount of the membrane Protein Fraction and process with Perfect-FOCUS kit. Follow the Perfect-FOCUS protocol. Process only as much protein as you need (i.e. 50-200µg protein/run). At the end of the Perfect-FOCUS protocol you will collect a protein pellet, suspend the pellet in the hydrated FPS Buffer (*See Preparation Before Use*) and run IEF/2D gel analysis.

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 Perfect-FOCUS 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., Manbison, 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

RELATED PRODUCTS

1. **FOCUS Protease Arrest (Cat # 786-108F)**: A protease cocktail specifically developed for sample preparation for 2D-studies and provides 95-98% inhibition of protease activity.
2. **PAGE Perfect (Cat #786-123)**: A kit for preparing sample for PAGE electrophoresis.
3. **FOCUS-Fast Silver (Cat # 786-240)**: Silver staining for protein gels, compatible for MassSpec analysis.
4. **Non-Interfering (NI) Protein Assay Kit (Cat #786-005)**: A protein assay that is free from interference of common laboratory agents including reducing agents, detergents, dyes, EDTA etc.
5. **RAPID-Stain (Cat #786-31)**: For staining protein in gels. RAPID-Stain only stains proteins, leaving clear background with high band visibility. Generally does not require de-staining.
6. **Tube-O-DIALYZER** - No loss dialyzer for small samples.

Note: For other related products, please visit our web site at www.GBiosciences.com or contact us.

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