



FOCUS™ Signal Proteins

INTRODUCTION

Signal Proteins: Caveolin-enriched membrane proteins and membrane proteins rich in cholesterol, glycolipids, and glycosylphosphatidylinositol (GPI) are generally not soluble in a wide range of non-ionic detergents. These proteins are believed to be involved in directing intracellular membrane traffic [1-3]. FOCUS™ -Signal Proteins kit is designed to fractionate caveolin-enriched membrane proteins, proteins rich in cholesterol, glycolipids, GTP-binding, and GPI-linked proteins. The extraction method involves solubilization and removal of hydrophilic proteins as well as hydrophobic (membrane) proteins soluble in non-ionic detergents. Signal Protein Extraction Buffer is a proprietary formulation of non-ionic detergents designed to efficiently extract and remove soluble proteins, leaving signal proteins as detergent insoluble fraction. After fractionation, the signal protein fraction is solubilized in FOCUS™ Protein Solubilization Buffer supplied with the kit. The signal protein 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# 786-250

FOCUS Signal Protein Extraction Buffer-I	[SPE Buffer-I]	50ml
FOCUS Signal Protein Extraction Buffer-II	[SPE Buffer-II]	50ml
FOCUS™ Protein Solubilization Buffer	[FPS Buffer]	25g
FOCUS™ Extraction Buffer	DILUENT- III	30ml

STORAGE CONDITION

Shipped at ambient temperature. Upon arrival store 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 an appropriate diluent. 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. 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 protease inhibitor cocktail in SPE Buffer-I to prevent protease activities during extraction procedure (see Related Products for protease inhibitor Protease Arrest™).
3. SPE Buffer-I & SPE Buffer-II- Before use, make sure the buffers are chilled, alternatively, place the buffers in ice-bath for 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 SPE Buffer-I.
For each 0.05ml of wet animal cell pellet, use approximately 0.2-0.3ml SPE Buffer-I
Yeast - for 0.05ml wet yeast pellet use 0.25ml SPE Buffer-I
Bacteria- for 0.05ml wet *E.coli* pellet use 0.25ml SPE Buffer-I
Plant - use 1ml SPE Buffer-I for each 1 gram 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 10-20 seconds and chill the suspension between ultra-sonic bursts. Disruption of cells depends upon the nature of cells. Animal cells are disrupted within 10 seconds. *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 Signal Protein Extraction Buffer-II [SPE 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 15 minutes.
4. Centrifuge the suspension at 20,000x g for 15 minutes at 4-5°C. Remove and discard the clear supernatant.
5. Suspend the pellet in 1/3 the volume of SPE Buffer-I used in the previous Step-1 and an equal volume of SPE Buffer-II. Repeat the Steps 3-4. Remove and discard the clear supernatant.
6. Collect the pellet, which contains Signal proteins. Suspend the pellet in 0.1-0.3ml FPS Buffer to solubilize the in-soluble Signal Protein Fraction. Vortex the suspension 4-5 times, 60 seconds each. Incubate for 10-15 minutes at room temperature, vortex the suspension periodically. Centrifuge 18,000xg for 10 minutes at 20-25°C and collect the clear supernatant. Re-extract any residual pellet with 1/3 the volume of FPS Buffer used in the previous step. Pool the supernatant with the previous supernatant.

Determine protein concentration (use Non-Interfering Protein Assay, *G Bioscience* Cat # 786-005).
Make appropriate dilution in the FPS buffer before running IEF/2D gels.

Debris: Depending on the source and the nature of the sample, some insoluble materials (debris) may be recovered after the pellet solubilization 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.

REFERENCES

1. *The glycosyl-phosphatidylinositol anchor of membrane proteins*, Low. M.G. (1989) *Biochemica et Biophysica Acta*, 988, 427-454.
2. *Caveolin, a protein component of caveolae membrane coats*. Rothberg. K.G., Heuser. J.E., Donzell. W. C., Ying. Y., Glenney. J. R., and Anderson. R.G. W. (1992) *Cell*, 68, 673-682
3. *Potocytosis: Sequestration and Transport of Small Molecules by Caveolae*. Anderson, R.G. W., Kamen. B. A., Rothberg. K. G., and Lacey. S. W. (1992) *Science*. 255,410-411.

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)**: Sufficient for 25 gels.
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. **FOCUS™ Protein Reduction-Alkylation (Cat.#786-229)**: The Reduction-Alkylation kit offers a simple two step method for reduction and alkylation of protein samples for 2D gel analysis.
7. **Tube-O-DIALYZER**: No loss dialyzer for small samples.

NOTE: For other related products, visit our web site at www.GBiosciences.com or call us at 1-800-628-7730.

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