G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

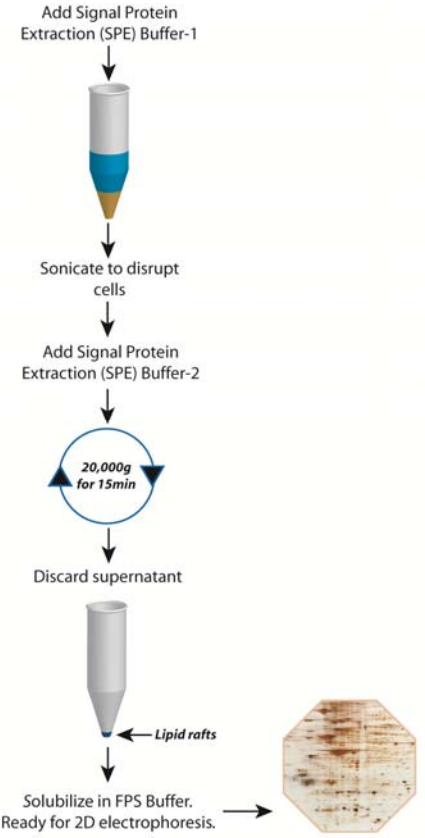
# FOCUS™ Signal Proteins

## (Cat. #786-250)

## INTRODUCTION

Signal Proteins: Caveolin-enriched membrane proteins and membrane proteins rich in cholesterol, glycolipids, and glycosyl-phosphitidylinositol (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).



## ITEM(S) SUPPLIED (Cat. # 786-250)

| Description | Size |
|---|---|
| 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 CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components as individually marked.

## 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. Add needed agents such as reducing agent, carrier ampholyte, and if necessary an appropriate protease cocktail.
2. If the inhibition of protease activity is required, add protease inhibitor cocktail in SPE Buffer-I to prevent protease activities during extraction procedure. We recommend our ProteaseArrest™ Protease Inhibitor Cocktail (Cat. # 786-108).
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.
   a. For each 0.05ml of wet animal cell pellet, use approximately 0.2-0.3ml SPE Buffer-I
   b. Yeast - for 0.05ml wet yeast pellet use 0.25ml SPE Buffer-I
   c. Bacteria- for 0.05ml wet *E.coli* pellet use 0.25ml SPE Buffer-I
   d. Plant - Use 1ml SPE Buffer-I for each 1gram plant tissue.
2. The sample to buffer volume ratio specified above is only a guide and may be adjusted depending on the scale of preparation.
3. Sonicate the suspension with an ultrasonic probe to break the cells and break down the genomic DNA.
   ***NOTE:*** *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.* D*isruption 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.*
4. 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.
5. Centrifuge the suspension at 20,000x g for 15 minutes at 4-5°C. Remove and discard the clear supernatant.
6. 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.
7. 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.
8. 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 supernatants.
9. Determine the protein concentration We recommend G-Biosciences' Non-Interfering Protein Assay (Cat. # 786-005).
10. Make appropriate dilution in the FPS buffer before running IEF/2D gels.

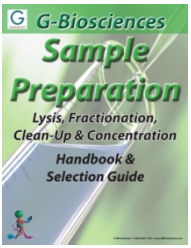
***NOTE:*** *Depending on the source and the nature of the sample, some insoluble materials (debris) may be recovered after the pellet solubilization steps. For solubilization of difficult-to-extract proteins, you may try the range of specialized FOCUS-Extraction Buffers we offer. Visit [www.GBiosciences.com](http://www.GBiosciences.com) for more information or contact our Technical Support.*

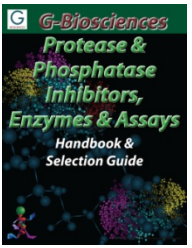
## REFERENCES


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

Download our Sample Preparation and Protease & Phosphatase Inhibitors, Enzyme & Assays Handbooks.




http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/
http://info.gbiosciences.com/protease-phosphatase-inhibitors-enzymes-assay-handbook

*For other related products, visit our website at www.GBiosciences.com or contact us.*

Last saved: 12/3/2012 CMH

*This page is intentionally left blank*


www.GBiosciences.com