



FOCUS™ - Soluble & Insoluble Protein Extraction

INTRODUCTION

FOCUS™ - Soluble & Insoluble Protein Extraction - Allows selective preparation of soluble (hydrophilic) proteins and insoluble (hydrophobic) proteins. The soluble and insoluble proteins may be further fractionated using our Fraction FOCUS kit, for more information visit www.GBiosciences.com.

ITEMS SUPPLIED

Cat # 786-247]

Soluble Protein Extraction Buffer	[SPE Buffer]	50ml
FOCUS™ Protein Solubilization Buffer	[FPS Buffer]	25g (Enough for 50ml)
FOCUS™ Extraction Buffer	DILUENT-III	30ml
Perfect-FOCUS™	[Cat # 786-124]	1 Kit

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.

ITEM NEEDED BUT NOT SUPPLIED

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

PREPARATION BEFORE USE

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 inhibitor cocktail.

Protease Inhibition- If the inhibition of protease activity is required; add a cocktail of protease inhibitors in SPE Buffer to prevent protease activities during extraction procedure (see Related Products for protease inhibitor Protease Arrest™).

PROTOCOL

- 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.
 Yeast - for 50µl wet yeast pellet use 0.4ml SPE Buffer.
 Bacteria- for 50µl wet *E. coli* pellet use 0.4ml SPE Buffer.
 Plant - use 2ml SPE Buffer 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.
- 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 30-40 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.
- Centrifuge the homogenate at 20,000xg for 30 minutes at 4-5°C to pellet the debris.
- Use a pipettor to remove the clear supernatant (without disturbing the pellet).
- Suspend the pellet in 1/4 the volume of SPE Buffer used in the previous Step-1. Sonicate the pellet once briefly (30 seconds). Repeat Steps 2-4. Collect the clear supernatant and pool with the first supernatant.
- Wash the pellet with 0.5ml SPE Buffer - Suspend the pellet in SPE Buffer, vortex for 60 seconds, and centrifuge at 20,000xg for 15 minutes at 4-5°C. Remove and discard the wash, the clear supernatant (OPTIONAL- wash may be saved or



pooled).

Mark the fractions as follows-

Clear Supernatant	Soluble Protein Fraction [Hydrophilic Fraction]
Cellular Debris & Pellet	Insoluble Protein Fraction [Hydrophobic Fraction]

Store both fractions at -70°C until used.

Processing “Soluble Protein Fraction” for IEF/2D analysis-

Determine protein concentration of the soluble protein fraction (Non-Interfering Protein Assay, *G-Biosciences* Cat# 786-005). For IEF/2D gel analysis, use an appropriate amount of the “Soluble Protein Fraction” and process with *Perfect-FOCUS™* kit. Follow the *Perfect-FOCUS™* protocol. Process only as much protein as you need (i.e. 50-200 μg protein /run). At the end of the *Perfect-FOCUS™* protocol you will collect a protein pellet, suspend the pellet in FPS Buffer and run IEF/2D gel analysis.

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 “In-Soluble Protein fraction” for IEF/2D analysis-

Suspend the in-soluble protein in 0.3-4ml FPS Buffer. Vortex the suspension 4-5 times, 60 seconds each, to solubilize the in-soluble protein. Centrifuge 20,000xg for 30 minutes at 15-20 $^{\circ}\text{C}$ and collect the clear supernatant.

Re-suspend any residual pellet in 1/4 the volume of FPS Buffer used in the previous step. Centrifuge 20,000xg for 30 minutes at 15-20 $^{\circ}\text{C}$ and collect the clear supernatant and pool the supernatant with the previous supernatant. Determine protein concentration of the solubilized In-Soluble Protein Fraction (Non-Interfering Protein Assay, *G-Biosciences* Cat # 786-005). Make an appropriate dilution in 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 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. Use *Perfect-FOCUS™* (Cat # 786-124) for cleaning and preparing sample for 2D gels.

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 samples for PAGE electrophoresis.
- 3. FAST-Silver (Cat # 786-30):** For staining proteins and Nucleic acids in acrylamide gels.
- 4. FOCUS-Fast Silver (Cat # 786-240):** Sufficient for 25 gels.
- 5. 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.

For other related IEF/2D/ Proteomic analysis products, visit our web site at www.GBiosciences.com

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