



BIOSCIENCES[®]

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FOCUS[™] - Mammalian Proteome

INTRODUCTION

FOCUS[™] - Mammalian Proteome kit extracts all of the proteins, including membrane as well as soluble proteins, from animal cells & tissues. The kit is supplied with a strong chaotropic extraction buffer to solubilize even the most difficult membrane proteins. After solubilization, the sample may be applied directly on IPG-Strips for IEF/2D analysis.

Note: For extraction of total proteome from bacteria, yeast and plant, refer to the following kits – FOCUS[™]-Bacterial Proteome (Cat# 786-258), FOCUS[™]-Yeast Proteome (Cat# 786-257) and FOCUS[™]-Plant Proteome (Cat# 786-259).

ITEMS INCLUDED

Cat # 786-246

FOCUS [™] Protein Solubilization Buffer [FPS Buffer]	25g (enough for 50ml)
FOCUS [™] Extraction Buffer [DILUENT- III]	30ml

STORAGE CONDITION

Shipped at Ambient Temperature. Store the kit components as individually marked upon arrival.

ITEMS 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 bottles 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.

PROTOCOL

1. For each 100 mg of animal tissues, use approximately 0.4-0.5ml **FPS Buffer**.
For each 0.05ml (~10 million cells) of wet animal cell pellet, use approximately 0.4-0.5ml **FPS Buffer**.

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. Centrifuge the homogenate at 20,000xg for 30 minutes at 20°C to pellet the tissue debris.
4. Use a pipettor to transfer the clear extract supernatant into a clean tube without disturbing the pellet.
5. Suspend any residual cell debris in 1/4 the volume of FPS Buffer used in the previous Step-1. Sonicate the suspension once briefly (30 second). Repeat Step 3. Collect the extract and pool with the first extract supernatant. Store total protein extract at -70°C until used.

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



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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 Tech 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. Visit www.GBiosciences.com for more information or contact our Technical Support.

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. Protease Arrest (Cat # 786-108):** A cocktail of protease inhibitors for use during protein extraction and purification. Protease Arrest inhibits a broad spectrum of serine, cysteine and metalloproteases as well as calpains.
- 3. PAGE Perfect (Cat. #786-123):** A kit for preparing sample for PAGE electrophoresis.
- 4. Perfect-FOCUS (Cat # 786-124):** A kit for preparing sample for 2D gels.
- 5. FAST-Silver (Cat # 786-30):** For staining proteins and Nucleic acids in acrylamide gels.
- 6. FOCUS-Fast Silver (Cat # 786-240):** Sufficient for 25 gels.
- 7. 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.
- 8. 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.
- 9. 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.
- 10. Tube-O-DIALYZER** - No loss dialyzer for small samples.

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