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A Geno Technology, Inc. (USA) brand name

FOCUS™ Mammalian Proteome

(Cat. # 786-246)

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.

ITEM(S) SUPPLIED (Cat. # 786-246)

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

STORAGE CONDITION

The kit is shipped at ambient temperature. Store the kit components as individually marked upon arrival.

ADDITIONAL ITEM(S) REQURED

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 the Step 3. Collect the extract and pool with the first extract supernatant. Store total protein extract at -70°C until used.
6. Determine protein concentration and make an appropriate dilution in FPS Buffer before running IEF/2D gels.

Debris:

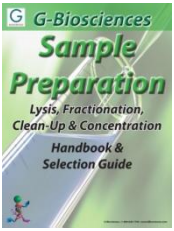
Depending on the source and the nature of the sample, some insoluble materials (debris) may be recovered after the extraction steps. For solubilization of difficult-to-extract proteins, you may try our range of specialized FOCUS™ Extraction Buffers.

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

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook

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

Last saved: 7/10/2014 CMH

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