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

A Geno Technology, Inc. (USA) brand name

FOCUS™ Insect Proteome

(Cat. # 786-360)



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INTRODUCTION

FOCUS™ Insect Proteome kit extracts the total proteome, including difficult to solubilize membrane proteins, from insect cells (i.e. Sf9 and Sf21). The kit is supplied with a strong chaotropic extraction buffer to solubilize proteins, which is compatible with IEF and 2D electrophoresis. This allows the sample to be applied directly to IPG-Strips for IEF and 2D analysis.

NOTE: For extraction of total proteome from mammalian cells, bacteria, yeast and plant, refer to the following kits: FOCUS™ Mammalian Proteome (Cat.# 786-246), FOCUS™ Bacterial Proteome (Cat.# 786-258), FOCUS™ Yeast Proteome (Cat.# 786-257) and FOCUS™ Plant Proteome (Cat.# 786-259).

ITEM(S) SUPPLIED (Cat. # 786-360)

Description	Size
FOCUS™ Protein Solubilization Buffer [FPS Buffer]	25g*
FOCUS™ Extraction Buffer [DILUENT III]	30ml

*Makes a final volume of 50ml with the addition 30ml DILUENT III

STORAGE CONDITION

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

ADDITIONAL ITEM(S) REQUIRED

Centrifuge, centrifuge tubes, reducing agent, alkylation agents, carrier ampholytes, and protease inhibitor cocktail (we recommend FOCUS ProteaseArrest™, Cat. # 786-108F).

PREPARATION BEFORE USE

1. Vigorously shake the FPS Buffer for 10-15 seconds.
2. For every 50µl wet insect cell pellet, weigh out 0.5gm FPS Buffer.
3. Immediately before use, add 550µl DILUENT III for every 0.5 gram FPS Buffer used and periodically vortex at room temperature until complete dissolved. Add needed agents such as reducing agent, carrier ampholytes, and if necessary an appropriate protease inhibitor cocktail.

NOTE: Do NOT store final FPS Solution; make fresh each time for optimal solubilization.

PROTOCOL

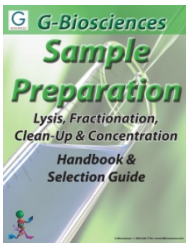
1. For each 50µl of wet insect cell pellet, use approximately 500µl final FPS Solution.
NOTE: *The sample to buffer volume ratio is a guide and may be adjusted depending on the scale of preparation.*
2. Sonicate the suspension at 4°C in an ice cold bath, ensuring no excess heating occurs. Use short bursts with short incubation times between bursts in ice-cold bath.
3. Centrifuge the homogenate at 20,000xg for 30 minutes at 20°C to pellet the cell debris.
4. Use a pipette to transfer the clear extract supernatant into a clean tube without disturbing the pellet.
5. Suspend any residual cell debris in ¼ volume of final FPS Solution. Sonicate and centrifuge as before. Pool supernatants.
6. Determine protein concentration and dilute sample with final FPS Solution.
NOTE: *We recommend Non-Interfering™ Protein Assay (Cat. # 786-005) as it is unaffected by the chaotropic FPS buffer.*
7. Store total protein extract at -70°C until used.
8. Depending on the source and the nature of the sample, some insoluble material (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 department.

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 department.

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.



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