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A Geno Technology, Inc. (USA) brand name

Fraction-FOCUS

(Cat. #786-168)

INTRODUCTION

The greatest challenge in protein discovery and the analysis of the proteome is the preparation of samples for 2D-gel analysis. In whole cell lysate, there are proteins that exist in a high dynamic range of concentration; consequently, abundant proteins mask identification of less abundant proteins. An effective proteome analysis will require separation of abundant proteins and enrichment of less abundant proteins, bringing the less abundant proteins into the dynamic range of detection. By fractionation, one simplifies a protein mixture by reducing the amount and the number of protein species to be loaded onto the gel matrix. Fractionation produces less crowded individual protein maps, simplifying analysis and interpretation.

G-Biosciences have developed a simple and reproducible method of serial fractionation of total cellular proteins called Fraction-FOCUS™. Fraction-FOCUS™ exploits the property of protein molecules to precipitate in response to the changes in solvent composition (i.e. ionic strength, pH, temperature). G-Biosciences has developed Fraction Precipitation Buffer for changing solvent composition in a step-wise to fractionate proteins.

The Fraction-FOCUS™ kit has been optimized to allow the fractionation of protein samples into a set of 7 fractions with no loss of protein. Fraction-FOCUS™ protocol is provided with the option to modify the use of Fraction Precipitation Buffer and create an additional set of distinct fractions in order to enhance the probability of discovering novel proteins. This kit is suitable for 10 separate preparations, with ~0.5ml protein extract in each set.

ITEM(S) SUPPLIED Cat # 786-168

Description	Size
Soluble Protein Extraction Buffer (SPE Buffer)	50ml
Fraction Precipitation Buffer (FP Buffer)	15ml
Universal Protein Precipitation Agent-I (UPPA-I)	2 x 30ml
Universal Protein Precipitation Agent-II (UPPA-II)	2 x 30ml
Orgosol™ Buffer	1 x 50ml
FOCUS™ Protein Solubilization Buffer (FPS Buffer)	25g
DILUENT-III	30ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store the kit at room temperature upon arrival. After first use, store the kit components as specified on the labels. The kit components are stable for 1 year.

ADDITIONAL ITEM(S) REQUIRED

Centrifuge, dialysis device (Tube-O-DIALYZER), Protease inhibitor cocktail (*FOCUS-Protease Arrest*™), Nuclease (FOCUS™-Nuclease™), 15ml tubes, Grinding resins (Molecular Grinding Resin) with Matching pestles & tubes.

PREPARATION BEFORE USE

- Transfer the Orgosol™ Buffer to -20°C 1-2 hours before use. Prepare FOCUS™ Protein Solubilization Buffer (FPS Buffer) according to the instructions on the label. Warm the Fraction Precipitation Buffer (FP Buffer) to room temperature and make sure that there is no crystal formation in the buffer.
- **Protease Inhibition** - If the inhibition of protease activity is required; add a cocktail of protease inhibitors *(FOCUS™ Protease Arrest™, see Related Products)* into the SPE Buffer.
- **Removal of Nucleic Acids** - If the removal of nucleic acids is required; add an appropriate amount of DNase and RNase into the SPE Buffer. Use nuclease cocktail FOCUS Nuclease™ *(see Related Products for **Longlife™**-Nuclease).*

PROTOCOL: PREPARATION OF SOLUBLE AND INSOLUBLE FRACTIONS

1. Sonicate the protein sample at 0-4°C in SPE Buffer. For 100mg tissue, 50mg wet cells and 50µl yeast or bacteria pellet use 0.4-0.5ml SPE Buffer. For 1gm plant tissue use 2ml SPE Buffer, to inhibit phenol related problems, add polyvinylpolypyrrolidone (PVPP) to the SPE Buffer.
   ***NOTE:** Sonication should be performed in a cold environment (ice cold bath) in bursts of 20-30 seconds. 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. Addition of glass beads in the yeast cell suspension greatly facilitates disruption of yeast cells.*
   Soft tissues may be homogenized using a pestle-tube homogenizer, electrical blender or a grinder. Molecular Grinding Resin (see related products) may be used for efficient grinding of tissues.
2. Centrifuge the homogenate at 20,000xg for 30 minutes at 4°C to pellet the cellular debris.
3. Use a pipette and transfer the clear supernatant to a clean 2ml tube without disturbing the pellet.
4. Suspend the pellet in 0.25 volumes of SPE Buffer used in Step-1. Sonicate the pellet once briefly (30 seconds). Repeat Steps 2-3. Collect the clear supernatant and pool with the first supernatant. Label "Soluble Fraction" and store on ice.
5. 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. Label the washed pellet as "Insoluble Fraction".
   ***NOTE:** For best results, fractionate the soluble proteins fractions within 2-3 hours. Storage and freezing of the soluble protein fraction may lead to precipitation of some soluble proteins.*

PROTOCOL: FRACTIONATION OF SOLUBLE FRACTION

The soluble fraction is serially fractionated with the stepwise addition of the Fraction Precipitation Buffer (FP Buffer).

Fraction-I

1. Transfer 0.5 ml of the "Soluble Fraction" to a 2ml microfuge tube labeled "Fraction 1".
2. In a drop-wise manner, add 0.1ml FP Buffer solution. Invert the tube 2-3 times and incubate on ice for 5 minutes.
   NOTE: *For smaller or larger sample volumes, the reagent volumes may be adjusted correspondingly.*
3. Centrifuge at 15,000xg for 10mins at 5°C. Transfer the supernatant to a clean 2ml tube and mark it as "Fraction 2".
4. Centrifuge "Fraction 1" again for a brief 5-10 seconds at 10,000xg and remove any residual supernatant. Store on ice.

Fraction-II

1. In a drop-wise manner, add 0.1ml FP Buffer to "Fraction 2". Invert the tube a few times and incubate on ice for 5 minutes.
2. Centrifuge at 15,000xg for 10mins at 5°C. Transfer the supernatant to a clean 2ml tube and mark it as "Fraction 3".
3. Centrifuge "Fraction 2" again for a brief 5-10 seconds at 10,000xg and remove any residual supernatant. Store on ice.

Fraction-III

1. Add 0.2ml FP Buffer to "Fraction 3". Invert the tube a few times and incubate on ice for 5 minutes.
2. Centrifuge at 15,000xg for 10mins at 5°C. Transfer the supernatant to a clean 2ml tube and mark it as "Fraction 4".
3. Centrifuge "Fraction 3" again for a brief 5-10 seconds at 10,000xg and remove any residual supernatant. Store on ice.

Fraction-IV

1. Add 0.3ml FP Buffer to "Fraction 4". Invert the tube a few times and incubate on ice for 5 minutes.
2. Centrifuge at 15,000xg for 10mins at 5°C. Transfer the supernatant to a clean 2ml tube and mark it as "Fraction 5".
3. Centrifuge "Fraction 4" again for a brief 5-10 seconds at 10,000xg and remove any residual supernatant. Store on ice.

Fraction-V

1. Add 0.8ml FP Buffer to "Fraction 5". Invert the tube a few times and incubate on ice for 5 minutes.
2. Centrifuge at 15,000xg for 10mins at 5°C. Transfer the supernatant to a clean 15ml tube and mark it as "Fraction 6".
3. Centrifuge "Fraction 5" again for a brief 5-10 seconds at 10,000xg and remove any residual supernatant. Store on ice.

Fraction-VI

1. Add 6ml UPPA-I to "Fraction 6". Invert the tube a few times and incubate on ice for 10 minutes.
2. Add 3ml UPPA-II. Vortex and incubate for 1 minute. Centrifuge at 18,000xg for 10 minutes at 5°C. Discard the supernatant.
3. Centrifuge "Fraction 6" again for a brief 5-10 seconds at 8,000xg and remove any residual supernatant.
4. Add 0.2ml UPPA-II on top of the pellet (be careful not to disturb the pellet). Centrifuge at 15,000xg for 10 minutes at 5°C. Remove and discard the clear supernatant (be careful not to disturb the pellet).
5. Add 0.1ml pure water on top of the pellet and vortex the tube for 30 seconds.
6. Add 5ml pre-chilled (-20°C) Orgosol™ Buffer and vortex the tube for 4-5 times, 30 seconds each. Incubate at -20°C for 30 minutes. Periodically vortex the tube to break up the pellet.
   NOTE: *The pellet will not dissolve*.
7. Centrifuge at 15,000xg for 10 minutes at 5°C. Discard the supernatant and invert the tube on a clean paper towel to remove residual Orgosol™ buffer. Allow the tube to air dry. Mark the tube as "Fraction 6"

Processing the Soluble Fraction Pellets

1. Suspend "Fraction 1-6" pellets in 0.1ml FPS buffer and vortex the suspension 5-6 times, 30 seconds each.
2. Prepare samples for 2D electrophoresis by dialyzing the suspensions against 2-3ml buffer of your choice for 5-6 hours with at least one change of the buffer.
   NOTE: *For best results, use Tube-O-DIALYZER™ for dialysis. We recommend dialysis against an appropriate IEF Buffer to remove all residual salt and make samples suitable for isoelectric focusing.*
3. Determine the protein concentration, we recommend *G-Biosciences* Non-Interfering Protein Assay. Make an appropriate dilution in an IEF compatible buffer of choice before running IEF/2D gels.
   NOTE: *The ideal protein concentration is dependent on gel size and detection method. Typical loadings are in the range of 50–150 µg per gel.*

Processing In-Soluble Protein Fraction for IEF/2D analysis

1. Resuspend the insoluble protein in 0.4ml FPS Buffer. Vortex the suspension 4-5 times, 60 seconds each, to solubilize the insoluble protein. Centrifuge 20,000xg for 15 minutes at 15-20°C and collect the clear supernatant.
2. Resuspend any residual pellet in 0.1ml FPS Buffer and centrifuge 20,000xg for 15 minutes at 15-20°C. Collect the clear supernatant and pool with the previous supernatant.
3. Determine protein concentration of the solubilized Insoluble Protein Fraction. Make an appropriate dilution in an IEF compatible buffer of choice before running IEF/2D gels.

   NOTE: *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, 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 (optional)

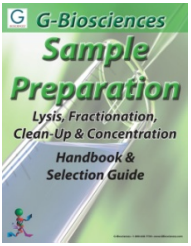
Depending on the nature of the samples (for example, plant leaves), it is sometimes necessary to clean the protein extracts or fraction before running IEF/2D analysis. We recommend *Perfect-FOCUS*™ for cleaning and preparing sample for 2D gels.

Optional Protocol

The sections using "Fraction Precipitation Buffer" will allow the fractionation of the soluble protein solutions into a set of 5 fractions. As some proteins have broad precipitation ranges, some proteins may appear in more than one fraction. Modifying the volume of FP buffer added at each stage can enrich a given protein into a single fraction. A 10-15% difference in the amount of FP Buffer used will significantly alter the fractionation profile. All modifications should be done empirically and once a suitable fractionation pattern is achieved the volumes added in subsequent experiments should be the same to maintain reproducibility.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



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

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