



FOCUS™ - Cytoplasmic & Nuclear Protein Extraction

INTRODUCTION

The kit **FOCUS™-Cytoplasmic & Nuclear Protein Extraction** has been developed for the enrichment of cytoplasmic and nuclear fractions from cultured cells and tissues for proteomic as well as expression and transport studies. Supplied with a strong chaotropic extraction buffer to solubilize both cytoplasmic as well as nuclear proteins for 2D gel analysis. The nuclear and cytoplasmic protein preparations are suitable for IEF & 2D gel electrophoresis.

ITEMS INCLUDED:

Cat# 786-248

SubCell Buffer-I	60ml
SubCell Buffer-II [3X]	30ml
SubCell Buffer-III	25ml
SubCell Buffer-IV	25ml
FOCUS™ Protein Solubilization Buffer [FPS Buffer]	25g
DILUENT- III	30ml
Perfect-FOCUS™ [Cat# 786-124]	1 kit

STORAGE CONDITION

The kit is shipped at ambient temperature. Store the *Perfect-FOCUS™* kit at room temperature. Store rest of the kit components at 4°C. The kit is stable for one year when stored unopened.

ITEMS NEEDED BUT NOT SUPPLIED

Syringes and 20 gauge needles or Wheaton Dounce homogenizer, centrifuge, centrifuge tubes, reducing agent, alkylation agents, carrier ampholytes, PBS, and protease inhibitor cocktail.

PREPARATION BEFORE USE

1. The kit is supplied with a FPS Buffer and DILUENT-III. 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 with DILUENT-III. Add needed agents such as reducing agent, carrier ampholyte(s), and if necessary an appropriate protease inhibitor cocktail.
2. Use aseptic technique when handling the SubCell Buffers. All SubCell buffers should be kept ice cold. Dilute appropriate volume of 3X SubCell Buffer-II to 1X with SubCell Buffer-I as needed (e.g. mix 2ml SubCell Buffer-I with 1ml SubCell Buffer-II). All centrifugation steps should be performed at 4°C except when mentioned in the protocols.

PROTOCOLS

A. Nuclear & Cytoplasmic Protein Extraction from Cultured Cells

This protocol is for processing 20×10^6 cells (or $\sim 100 \mu\text{l}$ wet cell pellet). It can be scaled up and down accordingly.

OPTIONAL: Add appropriate protease inhibitor cocktail (e.g. G-Biosciences Protease Arrest, Cat# 786-108) to SubCell Buffer- just before use.

1. Use fresh cells only. Pellet the harvested cells by centrifugation at $\sim 800 \times g$ for 1 minute. Carefully remove and discard the supernatant. Wash the cell pellet with 1ml ice cold PBS, centrifuge it as above and discard the supernatant.
2. Add 500 μl of ice cold SubCell Buffer-I. Gently vortex to suspend the cells and incubate on ice for 10 minutes.
3. Perform this lysis step on ice. Using a narrow opening (20 gauge) syringe needle, gently pull the suspension up and down 10-30 times. Alternatively, transfer cell suspension to ice cold Dounce homogenizer. Homogenize the cells on ice using tight pestle. Perform 5 to 20 strokes to lyse the cells effectively. Transfer the lysate to a microcentrifuge tube. Rinse Dounce homogenizer with 200 μl of SubCell Buffer-I and pool together. Invert the tube several times to mix.

NOTE: To check the cell lysis efficiency, spot 5 μl of cell lysate onto a glass slide, add coverslip and view under a phase-contrast microscope. Pulling times or strokes in the above lysis step are only guidelines. Mechanical force needed to lyse cells depends on cell types, the total number of the cells and hands on experience. Insufficient force will not lyse all the



cells and will lead to low cytoplasmic protein yield and contamination of cytoplasmic proteins to nuclear fraction, but will achieve cleaner cytoplasmic fraction with less nuclear contamination. Excess force may damage some nuclei and will lead to contamination of nuclear proteins to cytoplasmic fraction, but a cleaner nuclear fraction will be obtained.

This kit allows enrichment of nuclear and cytoplasmic fractions. Because of the nature of protein biosynthesis, protein transportation, and post-translational processing of proteins, cross-contamination of nuclear and cytoplasmic proteins is expected. Optimization of handling procedure may reduce cross-contamination.

4. Add 250 μ l 3X SubCell Buffer-II (350 μ l if Dounce homogenizer is used) and mix by inverting. This generates a 1X final concentration of SubCell Buffer-II.
5. Centrifuge the tube at 700x g for 10 minutes to pellet the nuclei. Transfer the supernatant (cytoplasmic fraction) to a new tube. The nuclear pellet can be washed with 500 μ l 1X SubCell Buffer-II and centrifuge again as above if further cleaning is not required.

NOTE: For further cleaning the nuclear fraction, see **Section B**.

B. Cleaning of the Nuclear Fraction

1. Resuspend the nuclear pellet in 300 μ l SubCell Buffer-III. Using a sharp pipet tip, remove the sticky lump if any. The lump is formed from dead cells and some lysed nuclei.
2. Centrifuge the tube at 700x g for 5 minutes and discard the supernatant. The nuclei containing pellet is clean enough for most purposes. If further cleaning is required, go to next step.
3. Pipet 300 μ l SubCell Buffer-IV to a 1.5ml centrifuge tube. Resuspend the pellet in 100 μ l SubCell Buffer-III. Carefully overlay the nuclei suspension on the surface of SubCell Buffer-IV.
4. Centrifuge the tube at 1,000x g for 10 minutes. Remove the supernatant and collect the very clean nuclear pellet in the tube.

C. Nuclear & Cytoplasmic Protein Extraction from Soft Tissues (liver or brain)

OPTIONAL: Delipidated BSA can be added to 1X SubCell Buffer-II to the concentration of 2mg/ml for removing free fatty acids prior to processing. An appropriate amount protease inhibitor cocktail also can be added to the 1X SubCell Buffer-II just before use.

1. Use a fresh tissue sample (obtained within one hour of sacrifice) kept on ice. Do not freeze.
2. Weigh approximately 50-100mg tissues. On a cooled glass plate, with the aid of a scalpel, mince the tissue into very small pieces.
3. Perform this step on ice. Transfer the minced tissue to an ice-cold Dounce tissue homogenizer. Add 10 volumes of 1X SubCell Buffer-II and using a loose-fitting pestle, disaggregate the tissue with 5-10 strokes or until the tissue sample is completely homogenized. Using a tight-fitting pestle, release the nuclei with 8-10 strokes. Do not twist the pestle as nuclei shearing may occur.
4. Stand on ice for 2 minutes. Transfer the homogenate to a centrifuge tube and leave large chunks of tissue in the homogenizer to be discarded. Centrifuge the lysate at 700x g for 5 minutes to pellet nuclei.
5. Transfer the supernatant (cytoplasmic fraction) to a new tube. Resuspend the nuclear pellet with 10 volumes of 1X SubCell Buffer-II and centrifuge again as step 4. The supernatant can be discarded as wash or pooled together as cytoplasmic fraction.

NOTE: For further cleaning of the nuclear fraction, see **Section B**.

D. Nuclear & Cytoplasmic Protein Extraction hard tissues (skeletal or heart muscle)

NOTE: For facilitating homogenization of the hard tissue, 0.25mg/ml Trypsin should be added to 1X SubCell Buffer-II. A concentrated BSA solution is needed to quench the proteolytic reaction after Trypsin treatment.

1. Use a fresh tissue sample (obtained within one hour of sacrifice) kept on ice. Do not freeze.
2. Weigh approximately 50-100mg tissues. On a cooled glass plate, with the aid of a scalpel, mince the tissue into very small pieces.
3. Suspend the sample with 8 volumes of 1X SubCell Buffer-II containing 0.25mg/ml trypsin in a 2ml centrifuge tube.
4. Incubate on ice for 3 minutes and then spin down the tissue at 1,000x g for 5-10 seconds in the centrifuge.

5. Remove the supernatant by aspiration and add 8 volumes of 1X SubCell Buffer-II containing 0.25mg/ml Trypsin. Incubate on ice for 20 minutes.
6. Add BSA Solution to a final concentration of 10mg/ml and mix. Spin down the tissue for a few seconds in the centrifuge.
7. Remove the supernatant by aspiration. Wash the pellet with 8 volumes of 1X SubCell Buffer-II without Trypsin, and spin down the tissue for a few seconds in the centrifuge.
8. Remove the supernatant by aspiration and add 8 volumes of the 1X SubCell Buffer-II without Trypsin.
9. Transfer the suspension to an ice-cold Dounce tissue homogenizer and using a loose-fitting pestle, disaggregate the tissue with 5-15 strokes or until the tissue sample is completely homogenized. Using a tight-fitting pestle, release the nuclei with 8-10 strokes. Do not twist the pestle as nuclei shearing may occur.
10. Stand on ice for 2 minutes. Transfer the homogenate to a centrifuge tube and leave large chunks of tissue in the homogenizer to be discarded. Centrifuge the lysate at 700x g for 5 minutes to pellet nuclei.
11. Transfer the supernatant (cytoplasmic fraction) to a new tube. Resuspend the nuclear pellet with 10 volumes of 1X SubCell Buffer-II and centrifuge again as step 10. The supernatant can be discarded as wash or pooled together as cytoplasmic fraction.

NOTE: For further cleaning the nuclear fraction, see **Section B**.

E. Solubilization of “Nuclei” for IEF/2D Analysis

1. Suspend nuclear fraction in 0.1-0.3 ml hydrated FPS Buffer. Vortex 4-5 times, 60 seconds each, to solubilize the nucleic proteins (Brief sonication or other mechanic force may be needed to break the genomic DNA).
2. Centrifuge 15,000xg for 15 minutes at 15-20°C and collect the clear supernatant.
3. Re-extract any residual pellet with 1/4 the volume of hydrated FPS Buffer used in the previous step. Centrifuge 15,000xg for 15-20 minutes at 15-20°C, collect the clear supernatant and pool the supernatant with the previous supernatant.
4. Determine protein concentration (use Non-Interfering Protein Assay, Cat# 786-105). Make an appropriate dilution in hydrated FPS Buffer before running IEF/2D gels.

F. Processing “Cytoplasmic Protein Fraction” for IEF/2D analysis

Determine the protein concentration of the cytoplasmic protein fraction (use Non-Interfering Protein Assay, Cat # 786-105). For IEF/2D gel analysis, use an appropriate amount of the “Cytoplasmic Protein Fraction” and process with Perfect-FOCUS kit. Follow the Perfect-FOCUS protocol. Process only as much protein as you need (i.e. 50-200µg protein /run). At the end of the Perfect-FOCUS protocol you will collect a protein pellet, suspend the pellet in hydrated FPS Buffer (*See Preparation Before Use*) and run IEF/2D gel analysis.

NOTE- The “Cytoplasmic Protein Fraction” may be directly mixed with FPS Buffer for running IEF/2D analysis. If the “Cytoplasmic Protein Fraction” is sufficiently concentrated, you may mix 1 part “Cytoplasmic Protein Fraction” with >20 parts FPS Buffer without seriously diluting the FPS Buffer.

RELATED PRODUCTS

1. **Microfuge tube & Matching Pestles (Cat. # 786-138P)**: microfuge tube with tight fitting pestle for processing small samples, such as extraction of nuclei, cytoplasmic protein, total lysate etc.
2. **FOCUS Protease Arrest™ (Cat. # 786-108F)**: A cocktail of protease inhibitors for use during protein extraction and purification. FOCUS- Protease Arrest™ inhibits a broad spectrum of serine, cysteine and metalloprotease as well as calpains.
3. **Non-Interfering Protein Assay™ (Cat. # 786-005 & 786-005P)**: A protein assay that is free from interference of common laboratory agents, including reducing agents, detergents, dyes, EDTA, etc.

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