FOCUS™ Cytoplasmic & Nuclear Protein Extraction

(Cat. # 786-248)

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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.

ITEM(S) SUPPLIED (Cat. # 786-248)

Description	Size
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
UPPA™ -I	15ml
UPPA™ -II	15ml
FOCUS™-Wash	2ml
OrgoSol Buffer™	50ml
SEED™	300µl
PerfectFOCUS™ Buffer-I	2ml
PerfectFOCUS™ Buffer-II	0.5ml

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.

ADDITIONAL ITEM(S) REQUIRED

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 20x10^6 cells (or ~100µl 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 ~800 x *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 500µl 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 200µl of SubCell Buffer-I and pool together. Invert the tube several times to mix.
   NOTE: *To check the cell lysis efficiency, spot 5µl 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 250µl 3X SubCell Buffer-II (350µl 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 500µl 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 300µl SubCell Buffer-III. Using a sharp pipette 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. Pipette 300µl SubCell Buffer-IV to a 1.5ml centrifuge tube. Resuspend the pellet in 100µl 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 chucks 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# 786-005). Make an appropriate dilution in hydrated FPS Buffer before running IEF/2D gels.

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

For IEF/2D gel analysis, use an appropriate amount of the membrane Protein Fraction, process only as much protein as you need (i.e. 50-200µg protein /run). Determine the protein concentration of the cytoplasmic protein fraction. We recommend using our Non-Interfering Protein Assay, Cat # 786-005).

> ***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.*

Important Notes

- Perform the entire procedure at 4-5°C (ice bucket) unless specified otherwise. Various incubation conditions must be strictly followed. Use 1.5ml microfuge tubes for processing protein samples. 0.5ml microfuge tubes are not recommended.
- Always position the microfuge-tubes in the centrifuge in the same orientation, i.e. cap-hinge facing outward. This will allow the pellet to remain glued to the same side of the tube during centrifugation and washing steps and minimize the loss of the protein pellets.
- Chill OrgoSol Buffer at –20°C for ~1hr or more before use

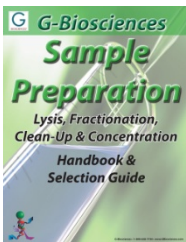
Protocol

1. Transfer 1-100µl protein solution (containing 1-100µg protein per sample) into a 1.5ml microfuge tube.
2. Add 300µl UPPA-I and mix well. Incubate at 4-5°C (ice-bucket) for 15 minutes.
3. Add 300µl UPPA-II in to the mixture of protein and UPPA-I, then vortex the tube.
   NOTE*: For larger sample size, use 3 volumes each of UPPA-I and UPPA-II for each volume of sample. See Appendix: Processing Large Samples.*
4. Centrifuge the tube at 15,000x g for 5 minutes to form a tight protein pellet.
5. As soon as the centrifuge stops, remove the tube from the centrifuge.
   NOTE*: Pellets should not be allowed to diffuse after centrifugation is complete.*
6. Carefully, without disturbing the pellet, use a pipette tip to remove & discard the entire supernatant.
7. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing out-ward. Centrifuge the tube again for 30 seconds. Use a pipette tip to remove the remaining supernatant.
8. Add 40µl of FOCUS-Wash on top of the pellet. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing out-ward.
   NOTE: *For larger sample size, add Wash 3-4 x times the size of the pellet.*
9. Centrifuge the tube again for 5 minutes. Use a pipette tip to remove and discard the Wash.
10. Add 25µl of pure water on top of the pellet.
    NOTE: *For large sample size, add water just enough to cover the pellet, i.e. a volume equal to the size of the pellet.*

11. Vortex the tube.
    NOTE: *Pellets do not dissolve in water*.
12. Add 1ml OrgoSol Buffer, pre-chilled at –20°C, and 5µl SEED.
    NOTE: *For large samples size, for each 0.1-0.3ml protein solution add 1ml OrgoSol Buffer. In addition, OrgoSol Buffer must be at least 10 fold in excess of the water added in Step 10.*
13. Vortex to suspend the pellet. It is important that the pellet is fully suspended in OrgoSol Buffer.
    NOTE: *Pellets do not dissolve in OrgoSol Buffer.*
14. Incubate the tube at –20°C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.
15. Centrifuge at 15,000xg for 5 minutes to form a tight pellet.
16. Remove and discard the supernatant. You will notice a white pellet in the tube. Air-dry the pellet. On drying, the white pellet will turn translucent.
    NOTE: *Do not over dry the pellets - parched dry pellets may be difficult to dissolve*.
17. Add an appropriate volume of hydrated FPS Buffer to suspend the pellet. Vortex the tube for 30 seconds. Incubate and vortex periodically until pellet is dissolved. Centrifuge and collect a clear protein solution and load on IEF gel.
    NOTE: *The Membrane Protein Fraction may be directly mixed with hydrated FPS Buffer for running IEF/2D analysis. If the Membrane Protein Fraction is sufficiently concentrated, you may mix 1 part Membrane Protein Fraction with >20 parts hydrated FPS Buffer without seriously diluting the FPS Buffer.*
    NOTE: *Hydrophilic proteins may also be processed for IEF/2D analysis using PrefectFOCUS kit as described above for the membrane protein fraction.*

RELATED PRODUCTS

Download our Sample Preparation Handbook



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

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