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FAST, ACCURATE, EASY- TO-USE

ProFEK™ – kit for isolation of nuclear, cytosol and membrane proteins using a bench top centrifuge

Proteins are dynamic and some proteins constantly move between different cellular compartments. Sub-cellular fractionation is necessary to identify where proteins are mainly localized and to gain a better understanding of their involvement in a particular disease or signaling pathway. Here we present a kit (ProFEK) and method that allows researchers to accomplish this essential step with a bench top centrifuge. ProFEK offers sufficient sub-cellular fractionation of proteins at the lowest cost per sample currently possible.

Proteins are dynamic in nature and their concentrations in different cellular regions vary depending on function, environmental factors and other stress experienced by the cells[1]. To understand this dynamic nature of the cells, it is imperative that researchers perform sub-cellular fractionation so that proteins predominant in cytosol, nuclear and membrane regions of the cells can be specifically targeted. However, a survey of current PubMed articles, reveals that most researchers perform whole cell lysis were the entire contents of the cells were digested without fractionation and analyzed. The biggest disadvantage is the loss of valuable information regarding the predominant localization of the proteins in the cells. For example, when studying drug interaction, based on the nature of the drug, certain proteins can be induced to cross the nuclear membranes and force the cells to either grow faster or induce death by apoptosis. Recently, it was demonstrated that in Melanoma cancers, as a result of mutation, ERK 1/2 gets continuously phosphorylated and the phosphorylated ERK 1/2 translocates to the nucleus and induces cellular growth leading to cancer and other neurodegenerative diseases[2]. This ground breaking discovery was made possible by sub cellular fractionation. Now there are drug which targets kinases which phophorylates ERK to prevent this translation.

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Another motivation for sub cellular fractionation is reducing the complexity of the biological samples. By fractionating proteins into nuclear, cytosol and membrane fractions the complexity of the sample is reduced drastically[3]. In a whole cell lysis, the protein solubilization is not fine tuned to the location of proteins. For example, majority of proteins in membrane are more hydrophobic compared to cytosolic fractions. Therefore, to identify the complete array of proteins in membrane, it is necessary to add detergents and chaotropic agents like urea to solubilize them. Reduction in sample complexity is also particularly useful when the objective is to identify low abundant proteins. As a result of fractionation, the proteins in each region will be selectively enriched, leading to successful protein identification by mass spectrometry.

In spite of the obvious benefits of sub-cellular fractionation, it is not widely utilized by many researchers. Some of the constraints include reproducibility, difficult protocols, cost and availability of the required equipment e.g. ultra-centrifuges. Amongst these constraints, the limited access to an ultra-centrifuge appears to be the biggest limitation for the performance of sub-cellular fractionation prior to sample analysis. Use of ultra-centrifugation devices needs training and constant maintenance. This adds both time and cost to the procedure. ITSI Bioscience's Protein Fraction Enrichment Kit (ProFEK) provides a validated, fast, and cost-effective enrichment kit for isolation and partial enrichment of proteins predominant in the membrane, cytosol, and nuclear regions of mammalian cells, and tissues without utilizing ultra centrifugation. The optimized ready-to-use reagents and procedure provided with

the kit allow for reproducible results and identification of unique region associated proteins. In this application note we illustrate the utilization of ProFEK to fractionate proteins from chicken heart and the profile of the isolated proteins on SDS-PAGE.

Protein Fractionation Enrichment

50 µg of heart tissue from chicken was used for the experiment. ProFEK was utilized to fractionate cellular proteins into cytosol, nuclear and membrane components using the manufacturer's recommendations. Tissue was chopped into smaller pieces and homogenized using cytosol buffer (10X the volume of tissue). Homogenized tissue was centrifuged at 3000g for 5 minutes at 4 °C. Supernatant is spun at 16000g for 10 minutes to clarify and the resulting supernatant is the cytosol enriched fractionation. The pellet obtained following the cytosol protein extraction was briefly washed and nuclear buffer was added and incubated for 30 mins with vortexing every 10 mins. After incubation, the solution was centrifuged for 10 mins at 16,000g. The supernatant is the nuclear protein enriched fraction. The resultant pellet from the nuclear protein extraction step was briefly washed and incubated with membrane buffer for 30 mins with vortexing every 10 mins. After centrifugation at 16,000g for 10 mins the supernatant obtained is the membrane enriched fraction. The same experiment was repeated with twice the amount of extraction buffer used in each step to determine the effect of extraction volume on total protein extraction.

SDS-PAGE Validation

Figure 1 shows the SDS-PAGE gel of protein profile obtained from cytosol, nuclear and membrane fractions. The lanes labeled "A" were the fractions obtained from utilizing 2X the recommended buffer and B 1X the buffer recommended. It was apparent that the three step process enriched different proteins to different extents, as evident from the protein bands on the gels. Also, the ratio of the volume reagent to the amount of sample impacted the total concentration of protein isolated. Image J software from NIH[4] was used to quantify the bands to access the efficiency of fractionation and unequivocally determine the difference in amount of protein extracted. The difference between the protein extracted in nucleus and membrane increased 18% and 11% respectively when twice the amount of buffer was used. However, cytosol proteins increased by 49% compared to using 1X volume of the extraction buffer. This shows the necessity to customize the protocol for each experiment to maximize fractionation and recovery. Figure 1 also shows the reproducibility of the protocol when comparing bands under section "A" vs. section "B".

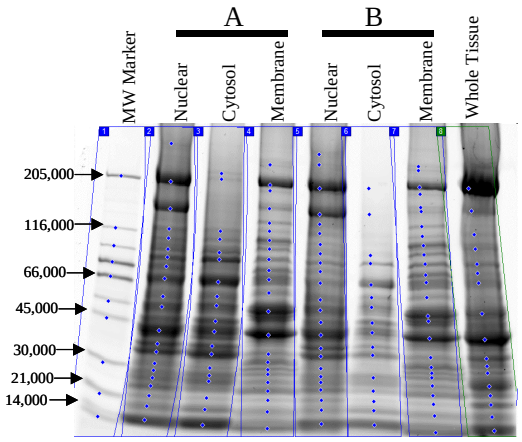

Figure 1: 1D-PAGE gel image of chicken heart tissue lysate fractionated with ITSIPrep ProFEK kit into Nuclear(1), Cytosol(2) and Membrane(3) protein enriched fractions. A and B represent the protein fractions obtained with 2x and 1x volume of solvent reactively, MW is molecular weight marker and WT is Whole Tissue

In conclusion, ITSI – Biosciences offers a validated kit for fractionation of proteins into nuclear, cytosol and membrane fractions using a bench top centrifuge. ProFEK utilizes ready-to-use reagents and easy-to-follow protocol to perform sub-cellular fractionation of proteins. This will enrich for proteins with specialized functions in different regions of the cell, reduce the complexity of samples and assist with identification of low abundant proteins. This kit can be utilized for both cells and tissues. The assay needs only 50 to 100 µg of total proteins, offers reproducible results and eliminates the guess work related from prepared reagents and location of proteins. This kit will aid shotgun proteomics and identification of low abundant cytosol, nuclear or membrane proteins. The low cost per sample combined with a simple-to-follow protocol makes this kit ideal for any proteomics, biochemistry or cell biology laboratory where there is interest to precisely determine whether proteins of interest are predominantly localized in the nuclear, cytosol or membrane regions.

References:

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