ITSIPrep™ Kits for Two – Dimensional Difference in Gel Electrophoresis

Successful Two-Dimensional Difference in Gel Electrophoresis (2D-DIGE) is dependent on the use of good quality reagents and validated protocols. Poor quality reagents and inadequate protocols will result in poor quality data, lack of reproducibility and waste of samples, time and hence money.

First reported by John Minden and colleagues in 1997 [1] and introduced commercially about 10 years ago by Amersham Biosciences (now GE Healthcare), Two Dimensional–Difference In Gel Electrophoresis (2D-DIGE) is a gel-based approach for comparative proteomics using fluorescent tags. Distinct florescent tags e.g. Cy 3, 5 and 2 are used to label samples and a universal internal standard prior to 1st/2nd dimension electrophoresis. An automated software program is used to detect, quantify and annotate differentially expressed proteins. GE Healthcare offers an integrated solution including, Cy Dyes, robotic spot pickers/digesters, Spot Handling Workstation and software [2]. Data obtained after 2D-DIGE experiment may include information on identity of the protein, pI, molecular weight and post translational modifications. 2D-DIGE offers all the advantages of 2D-PAGE and overcomes the inherent disadvantage of variation and reproducibility problem in a 2D-PAGE [2]. The 2D-DIGE process is still emerging and gaining wide acceptance. The scientists at ITSI-Biosciences had very early access to the 2D-DIGE technology; working closely with Amersham Biosciences in the early days of commercialization in both promoting and validating the technology [3-6], and pioneering some of the key modifications e.g. recommending the switch to the Typhoon Digital Imager as the scanner for capturing 2D-DIGE images rather than the originally supplied scanner which bleached 2D-DIGE gels. ITSI-Biosciences has developed a variety of complementary kits to streamline and standardize the upstream sample preparation steps prior to 2D-DIGE (Figure 1).

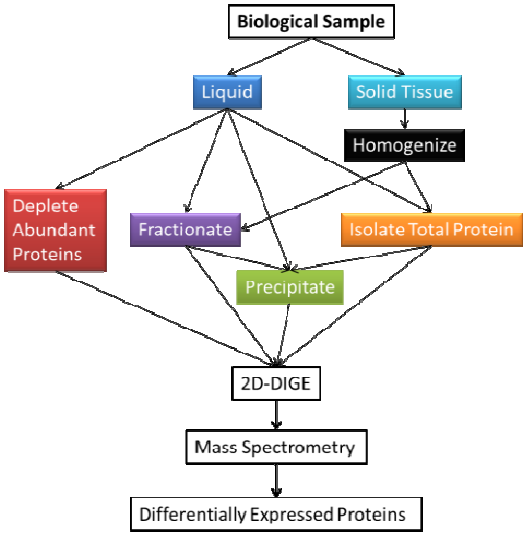

Figure 1: A successful 2D-DIGE experiment depends on the use of good quality reagents and protocols during the protein isolation, fractionation and precipitation steps.

Protein Extraction

The first step in a proteomics workflow processes is often total protein extraction from tissue or cells. This step requires the use of carefully standardized and reproducible procedures to ensure that good yield of proteins is consistently isolated without protein degradation. The ideal method must produce proteins suitable for the desired downstream

Kutralanathan Renganathan[1], Stephen Russell[1], Florentina Mayko[1], Steven Wolfe[1], Marissa Brainard[1], Stella B.Somiari[2], Richard I. Somiari[1*]

[1]ITSI-Biosciences, Johnstown, PA, USA; [2]Windber Research Institute, Windber, PA, USA. *Correspondence: Dr Richard Somiari. Email: Richard@itsibio.com.

application, while being reliable, convenient, fast, easy to perform and cost effective. 2D-DIGE is extremely sensitive to impurities, pH, water quality, and quality of buffers. Poor quality reagents negatively impact 2D-DIGE. The ITSI-Biosciences kits developed for 2D-DIGE offer optimized buffers and validated standard operating procedures that allow the isolation of total proteins from tissue and cell lines, fractionation of proteins and precipitation of proteins. The kits allows for a smooth transition from the protein isolation step to the downstream analytical processes often without the need for a buffer exchange.

Total Protein Isolation Kit for 2D-DIGE

The ITSIBIO ToPI-DIGE kit (K-0010) first introduced in 2005 in response to request from clients is the first validated kit specifically developed for isolation of proteins prior to 2D-DIGE. It contains a set of optimized reagents and standard operating procedures that allow research scientists to easily, conveniently and reproducibly isolate total proteins from human and non-human cell lines, solid tissue and tumor biopsies prior to 2D-DIGE [Figure 2]. It allows a smooth transition from protein isolation to downstream 2D-DIGE process without the need for time consuming and error prone buffer exchange.

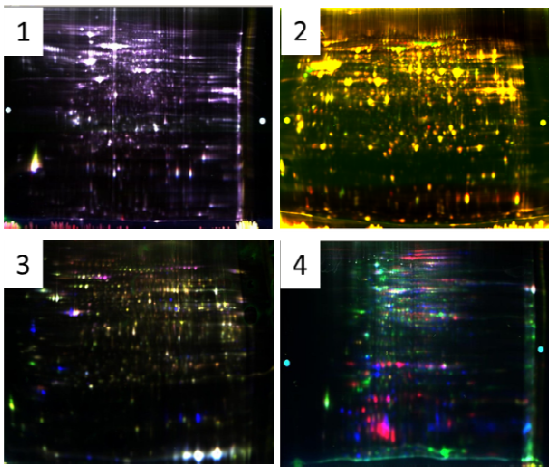

Figure 2: 2D-DIGE images obtained after total protein isolation with *ITSI* ToPI-DIGE protein isolation kit. 2D-DIGE was performed with IPG strips (pH 3-10) and 12.5% PAGE gels. Samples are 1) Human, 2) Monkey, 3) Mice and 4) fungus.

Plasma / Serum Protein Preparation Prior to 2D-DIGE

Human plasma / serum proteomics represents a unique proteome in many aspects. But one major difference is its wide dynamic range. This dynamic range is partly attributed to the Human serum albumin (66 KDa) that typically accounts for more than 65% of the total protein present in plasma and serum samples. High albumin concentration obscures the detection of low abundance plasma and serum proteins, and also Albumin (Alb) may distort lanes during electrophoresis if large amount of total protein is loaded. Albumin can also mask other proteins that migrate around the 50-70 kDA thereby preventing their detection. ITSIPrep ASKc (K-0012) is a validated spin column based protocol, and ASKs (K-0013) is a validated solvent based protocol for depletion of Alb from serum and plasma samples prior to analysis of the albumin enriched fraction (Alb+) and/or albumin depleted (Alb-) fraction. More than 90% of albumin is depleted from whole serum using either ITSIPrep ASKc Kit or ASKs Kit (Figure 3). Treated samples can be analyzed by electrophoresis (1D and 2D-DIGE), mass spectrometry and western analysis. These low cost alternatives to the relatively more expensive antibody/affinity column based depletion methods are useful for examining the validity of abundant protein removal as a strategy to reveal low abundant proteins prior to making huge investments in expensive methods.

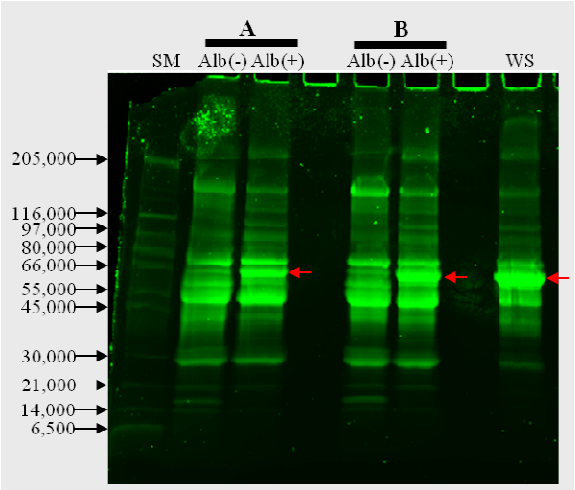

Figure 3: 1D-SDS PAGE (4 - 20 % gradient) gel image of human serum. SM is size marker and WS is Whole serum. "A" and "B" are replicates. The lanes marked Alb(-) and Alb(+) contain Albumin depleted and Albumin enriched fractions respectively.

Urine Protein Isolation Prior to 2D-DIGE

Urine proteomics is of interest for both biomarker discovery and for testing efficacy of biologics in pharmaceutical industries [7]. Our ITSIPREP™ ToPI-U and standardized operating procedure are developed exclusively for isolation and concentration of proteins from Urine prior to downstream analysis e.g. by electrophoresis (1D, 2D and 2D-DIGE) and western analysis. The ToPI-U mini kit contains optimized and ready-to-use reagents and concentration devices for processing of up to 10 urine samples. The use of this kit provides a standard method for processing urine samples after collection to isolate urine proteins. The result is that researchers can store/bank small vials/tubes rather than large volumes of urine thereby using scarce freezer space more efficiently.

Protein Fraction Enrichment Prior to 2D-DIGE

Cells contain complex mixture of proteins localized in different compartments, e.g. membrane, nucleus and cytoplasm. Therefore, to identify the most complete array of proteins in a cell, it may be necessary to do sub-cellular fractionation. This also reduces the sample complexity and aids in the analysis of proteins that occur at low concentrations in the cell. An ideal method for sub-cellular fractionation should be reproducible, simple, convenient and cost effective.

ITSIPrep ProFEK (K-0015) Protein Fraction Enrichment Kit (ProFEK) provides a validated, fast, and cost-effective system for partial isolation and concentration of proteins predominant in the membrane, cytosol, and nuclear regions of mammalian cells and tissues without sucrose gradient centrifugation [Figure 4].

The optimized reagents provided with the kit allow for reproducible results and display of unique region associated proteins that occur at low abundance. The extracted membrane, nuclear, and/or cytoplasmic protein fractions are suitable for SDS-PAGE, 2D-PAGE, 2D-DIGE, western blotting and mass spectrometry.

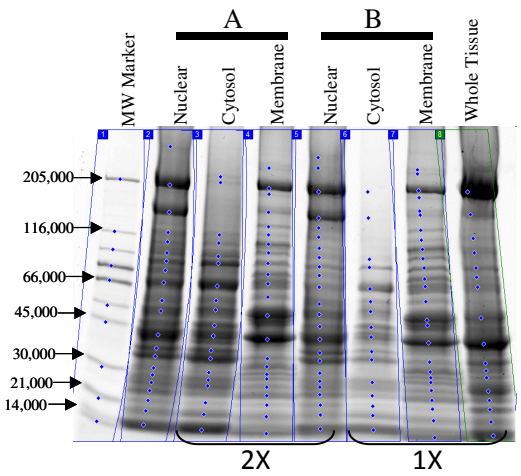

Figure 4: 1D-SDS PAGE gel image of heart tissue lysate fractionated with *ITSIPrep* ProFEK kit into Nuclear, Cytosol and Membrane proteins. A and B represent the protein fractions obtained with 2X and 1X volume of solvent respectively

Total Protein Precipitation Prior to 2D-DIGE

Protein precipitation may be necessary prior to 2D-DIGE. This step is often required for concentration of proteins and when buffer exchange is necessary. To be useful, the procedure must be reproducible, permit high protein recovery without changing the balance of protein levels, be simple to perform and cost effective. The easy-to-use Protein Precipitation Kit (ToPREP, K-0016) is specifically formulated for precipitation of proteins from solutions with higher than 90% recovery in general. The kit is unique because it contains ProPreCip™ a protein precipitation booster, optimized reagents, and standard operating procedure. This allows research scientists to easily, conveniently, and reproducibly precipitate total proteins or perform buffer exchange. The precipitated protein is suitable for 1D-PAGE, 2D-PAGE, 2D-DIGE, Western analysis and mass spectrometry.

Conclusion

Taken together ITSI Biosciences has developed a set of unique kits validated for sample preparation prior to 2D-DIGE. The kits help to streamline and standardize the up-stream sample preparation steps including protein isolation, fractionation, precipitation and depletion (Figure 5), leading to faster experiments and more reproducible results. As these are ready-to-use kits they are convenient to use and more cost effective.

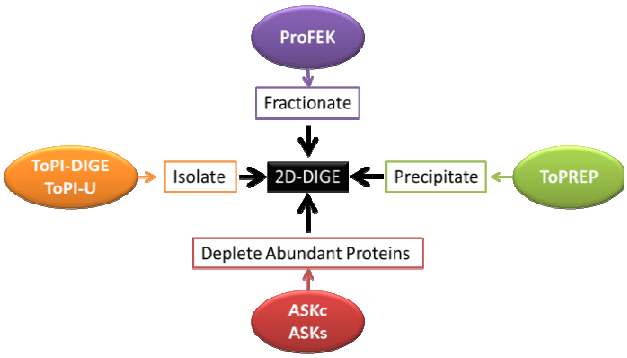

Figure 5: Sample preparation kits have been developed and validated for the 2D-DIGE process. The use of these kits streamlines and standardizes the upstream sample preparation step prior to the 2D-DIGE and allows researchers to take a full advantage of this powerful gel-based protein expression profiling technology.

References

1. Unlü M et al, *Electrophoresis*. 1997, 18(11):2071-7.
2. Somiari RI, et al, *Proteomics*. 2003. 3(10):1863-73.
3. Brzeski H et al. *Biotechniques*. 2003 Dec; 35(6):1128-32.
4. Somiari RI et al, *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005.5;815(1-2):215-25.
5. Boyiri T et al. *Int J Oncol.* 2009. 35(3):559-67
6. Sinha R et al. *Cancer Epidem Biomar* Prev. 2010. 19(9):2332-40.
7. Kentsis, A. (2011), *Pediatr Int*. 2011. 53: 1–6