## SAMPLE PREPARATION FOR 2D GEL ELECTROPHORESIS

Many of the chaotropic reagents, surfactants and denaturing agents used in the solubilization of proteins are incompatible with 2D electrophoresis. In addition, many other interfering agents are released during protein solubilization, such as lipids, polysaccharides and, in the case of plants, lignins, polyphenols, tannins, alkaloids, etc. 2D gel analysis separates proteins based on their charge and molecular weight and many of the interfering compounds affect these properties, making it difficult to identify novel proteins due to incorrect molecular weights and pls.

G-Biosciences has developed several products to aid researchers in the generation of 2D electrophoresis compatible samples.

## METHOD

Samples of human Jurkat cells, mouse liver, and spinach leaves were prepared by the following methods.

Sample-1 (Control): 5x10<sup>6</sup> Jurkat cells were suspended in FOCUS<sup>TE</sup> Extraction Buffer-V; a urea, thiourea & CHAPS buffer (Cat. #786-219) and were lysed by sonication. Sample-2: 5x10<sup>6</sup> Jurkat cells were suspended in PBS with 5mM DTT.

Sample-3:  $5x10^6$  Jurkat cells were lysed in SDS-PAGE sample loading buffer containing 0.1M Tris, 2%SDS,  $\beta$ -mercaptoethanol, and 0.001% bromophenol blue. Sample-4: Mouse liver protein was eluted from an ionexchange column using 1M NaCl. Sample-5: 50mg spinach leaves were ground up and extracted in SDS-PAGE buffer.

Protein concentration of each sample was determined using *Non-Interfering* Assay<sup>™</sup> (*NI*<sup>™</sup> Protein Assay, Cat. # 786-005).

G-Biosciences' *Perfect*-FOCUS<sup>™</sup> was used to remove 2D electrophoresis interfering agents. 50µg protein from samples 2-4 was processed according to *Perfect*-FOCUS<sup>™</sup> protocol. Briefly, protein samples were treated with UPPA- reagents, a proprietary precipitation agent. Protein pellets were collected by centrifugation in a microcentrifuge tube and interfering agents were washed away with Orgosol buffer provided with the kit. Clear white protein pellets were recovered. Protein pellets from all four samples were suspended and dissolved in 100µl Extraction Buffer-V.

An alternative to precipitation is dialysis. In a separate experiment, 50µg protein from samples 2 and 3 were dialyzed in a Tube-O-DIALYZER<sup>™</sup> (4000 mol. wt cut-off) according to the protocol. In brief, samples were transferred into the tubes, and the dialyzing caps were repositioned on the tube. Tube-O-DIALYZER<sup>™</sup> was positioned in a dialysis tank and dialyzed against Extraction Buffer-V (Figure 1).



Figure 1: Tube-O-DIALYZER<sup>™</sup> schematic.

2D electrophoresis was performed using IPG-strips, 7 cm in length and pH 3-10. After 12 hours of hydration, focusing was performed for 5 hours. Upon completion of the first dimension electrophoresis, the IPG-strips were equilibrated in FOCUS<sup>™</sup> Equilibration Buffer (Cat. #786-224). Second dimension SDS-PAGE was performed in 4-20% gradient gels. Gels were developed using FOCUS<sup>™</sup> FAST*silver*<sup>™</sup> stain (Cat. #786-240).

As previously stated, protein samples containing high concentrations of salts, SDS, and plant products are not suitable for running first dimension iso-electric focusing. Below is a summary of the results after comparison to the control (sample 1) (data not shown) 2D electrophoresis gel.

Sample-2 contained high salt concentration; following treatment with *Perfect*-FOCUS<sup>™</sup> a salt-free protein sample was obtained that produced a 2D gel comparable to the control.

Sample-3 contained the ionic detergent SDS, which severely impedes isoelectric focusing on IPG-Strips. Dialysis in a Tube-O-DIALYZER<sup>™</sup> removed SDS, making the sample suitable for 2D gel analysis (Figure 2). The gel was comparable to the control.



Figure 2: Sample-3 containing SDS was dialyzed in a Tube-O-DIALYZER  $\Tilde{\texts}$  before running a 2D gel.

Sample-4 contained an excessive concentration of salt (1M NaCl) and was not suitable for electrophoresis.

Cleaning the sample with *Perfect*-FOCUS<sup>™</sup> yielded a protein sample free of salt and produced a 2D gel comparable to the control.





Sample-5: The spinach leaf extract contained SDS and a high concentration of pigments and other plant products that interfere with 2D gel analysis. A protein sample free from the interfering agents was generated after treatment with *Perfect*-FOCUS<sup>™</sup> and produced a suitable 2D gel map (Figure 3).



Figure 3: Spinach leaves proteins after cleaning with Perfect-FOCUS<sup>™</sup>

## CONCLUSION

Cleaning samples prior to 2D electrophoresis is important for the removal of interfering agents. *Perfect*-FOCUS<sup>™</sup> allows removal of salt, detergents, and other agents from the sample without any adverse effects. Similarly, Tube-O-DIALYZER<sup>™</sup> allows quick removal of interfering agents. Both of these kits may be used for cleaning samples for 2D gels. After cleaning the sample, the 2D gel produced spot distribution similar to the control and the protein spots are suitable for mass spectrometry analysis.

## **ORDERING INFORMATION**

Cat. #	Description/Size
786-124	Perfect-FOCUS <sup>™</sup> / 50 Preps (1 kit)
786-141-1K	Tube-O-DIALYZER <sup>™</sup> , Micro (up to 250µl), 1kDa MWCO / 50
786-141-4K	Tube-O-DIALYZER <sup>™</sup> , Micro (up to 250µl), 4kDa MWCO/ 50
786-141-8K	Tube-O-DIALYZER <sup>™</sup> , Micro (up to 250µl), 8kDa MWCO / 50
786-141-15K	Tube-O-DIALYZER <sup>™</sup> , Micro (up to 250µl), 15kDa MWCO / 50
786-141-50K	Tube-O-DIALYZER <sup>™</sup> , Micro (up to 250µl), 50kDa MWCO / 50
786-142-1K	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 1kDa MWCO / 50
786-142-4K	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 4kDa MWCO / 50
786-142-8K	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 8kDa MWCO / 50
786-142-15K	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 15kDa MWCO / 50
786-142-50K	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 50kDa MWCO / 50
786-142-1T	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 1kDa MWCO / trial size
786-142-4T	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 4kDa MWCO / trial size
786-142-8T	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 8kDa MWCO / trial size
786-142-15T	Tube-O-DIALYZER <sup>™</sup> , Medi (up to 2.5 ml), 15kDa MWCO / trial size
786-143-1K	Tube-O-DIALYZER <sup>™</sup> , Mixed, (Medi & Micro), 1kDa MWCO / 50
786-143-4K	Tube-O-DIALYZER <sup>®</sup> , Mixed, (Medi & Micro), 4kDa MWCO / 50
786-143-8K	Tube-O-DIALYZER <sup>™</sup> , Mixed, (Medi & Micro), 8kDa MWCO / 50
786-143-15K	Tube-O-DIALYZER <sup>™</sup> , Mixed, (Medi & Micro), 15kDa MWCO / 50
786-143-50K	Tube-O-DIALYZER <sup>™</sup> , Mixed, (Medi & Micro), 50kDa MWCO / 50
786-027-4K	Tube-O-Reactor <sup>™</sup> 4kDa MWCO
786-027-8K	Tube-O-Reactor <sup>™</sup> 8kDa MWCO
786-027-15K	Tube-O-Reactor <sup>™</sup> 15kDa MWCO
786-145A	Tube-O-Array <sup>™</sup> (Tray, mini dialysis cups, floats, 100 glass stirring balls)

