Total Protein Profiling Employing a Novel Protein Fraction Method Combined with Tandem Mass Tag Labeling

**INTRODUCTION**

Accurate and reproducible quantitation of cellular proteins is a critical component in the study of cellular phenotypes and it underlies the interpretation of significant alterations of protein translationally regulated proteins. A common set of experimental constraints exist among these studies, and in addition, experiments vary in the labeling methods and sample preparation. Combining peptide-centric methods, such as MALDI-TOF-MS, and SILAC, 2DE, DIGE, or iTRAQ can be used to perform protein quantitation and identify altered proteins. However, smaller changes are not captured when these methods are employed. The use of TMT tags can improve the accuracy of these methods by providing a reproducible manner.

**METHODS**

Cells were maturated to different conditions (DMSO, SU11274, and Staurosporine) for 24 hours. Equal amounts of total protein from each condition were isolated and purified. Cysteine residues of protein from each sample condition were labeled with iodoacetamide. For the 300 μg of pooled proteins from the three experimental conditions (DMSO, SU11274, and Staurosporine) were labeled on cysteine residues and subjected to size based fraction and pre-programmed elution. 500 pg of proteins from the three experimental conditions (DMSO, SU11274, and Staurosporine) were labeled on cysteine residues and subjected to size based fraction and pre-programmed elution. 500 pg of proteins from the three experimental conditions (DMSO, SU11274, and Staurosporine) were labeled on cysteine residues and subjected to size based fraction and pre-programmed elution. 500 pg of proteins from the three experimental conditions (DMSO, SU11274, and Staurosporine) were labeled on cysteine residues and subjected to size based fraction and pre-programmed elution. 500 pg of proteins from the three experimental conditions (DMSO, SU11274, and Staurosporine) were labeled on cysteine residues and subjected to size based fraction and pre-programmed elution.

**CONCLUSIONS**

In combination with TMT labeling, data based fraction allows for more comprehensive and qualitative view of the proteins.

**REFERENCES**


(1) Cell Signaling Technology, Inc., Danvers MA 01923, (2) Sage Science, Inc., Beverly, MA 01915


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