Mass Spectrometric Quantitation of Differentially Expressed Protein From Stable Isotopically Labeled CHO Cell Media

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Introduction
Recent approaches in analytical biochemistry have made use of stable isotope labeling techniques for comparative protein analysis. Several methods of isotope incorporation have been explored including metabolic labeling of CHO cells and incorporation of fully labeled amino acids in medium containing amino acids labeled with stable isotopes. MALDI-TOF-MS coupled with SI is a powerful technique for relative quantitation and can be used to obtain differential protein expression data for performing expression profiling. The high purity of modern SI compounds minimizes isotopic contamination resulting in high-quality quantitative data. The option of using an essential amino acid such as lysine with both nitrogen and carbon SI labels greatly facilitates quantitation.

Materials
- Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 (10040-065, Sigma-Aldrich)
- Fetal Bovine Serum (FBS) (17500-095, Sigma-Aldrich)
- Radio-immunoprecipitation Assay (RIPA) Buffer (89901, Sigma-Aldrich)
- Protease Inhibitor Cocktail (58714, Sigma-Aldrich)
- Tryptic Profile in Gel Digestion (G2D) Kit (2275, Sigma-Aldrich)
- radioimmunoprecipitation assay buffer (89901)
- ProteoMass™ Peptide MALDI-MS Calibration Kit (MS-CAL2, Sigma-Aldrich)
- ProteoMass™ Guanidination Kit (MS0100, Sigma-Aldrich)
- ProteoMass™ Red Anti-FLAG M2 Affinity Gel (AF0416, Sigma-Aldrich)
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Methods
- Affinity purification was successful in capturing the tagged proteins using the EZview Red ANTI-FLAG M2 Affinity Gel.
- Stable isolate (SI) labeled media with either dialyzed or non-dialyzed FBS was capable of supporting cell growth.
- Cells were used efficiently for affinity purification while protease activity was minimized using RIPA buffer containing protease inhibitors.
- Affinity purification was successful in capturing the tagged proteins using the EZview Red ANTI-FLAG M2 Affinity Gel.
- Quantitation of the native and SI labeled proteins was performed by SDS PAGE. The gel showed elution of the FLAG-tagged protein.

Results
- Stable isolate (SI) labeled media with either dialyzed or non-dialyzed FBS was capable of supporting cell growth.
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Discussion
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- Quantitation of the native and SI labeled proteins was performed by SDS PAGE. The gel showed elution of the FLAG-tagged protein.
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