Applications of Mass Spectrometry Targeted Assays for Quantitative Analysis of Cancer Signaling Proteins

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ABSTRACT

Targeted mass spectrometry (MS) assays are emerging as quantitative tools for analysis of cancer signaling proteins. These methods have enabled high throughputs and low limits of detection suitable for clinical use. However, many assays require improvements in linearity, robustness, and multiplexing capabilities for comprehensive analysis of complex biological samples. The Thermo Scientific SureQuant™ Pathway panels have been applied, which utilize an optimized multiplex MS workflow. From the standard curve, all target peptides were monitored with <20% CV, 3 orders of magnitude dynamic range, linearity (R² ≥ 0.98), and quantitation for 12 samples measured in triplicate under 20 minutes. Kit performance was evaluated through Suitability Standard, AQUA Ultimate Heavy and/or AQUA Ultimate Light Peptides, and verified MS instrument and data workflow. From the standard curve, all target peptides were monitored with <20% CV, 3 orders of magnitude dynamic range, linearity (R² ≥ 0.98), and quantitation for 12 samples measured in triplicate under 20 minutes. Kit performance was evaluated through Suitability Standard, AQUA Ultimate Heavy and/or AQUA Ultimate Light Peptides, and verified MS instrument and data workflow. Quantitative measurement of alterations in the expression of pathway proteins and post-translational modifications (PTMs) is essential for understanding the role of cancer signaling in disease development and response to therapy. Here, we report the development of quantitative MS assays for the AKT/mTOR and ERBB/EGFR pathways by utilizing a unique approach for multiplexing and flexible target selection.

INTRODUCTION

Mass spectrometry is a powerful tool for the analysis of complex biological samples. The Thermo Scientific SureQuant™ Pathway panels are amenable to diverse sample sources and allowed identification of target proteins from cellular and tissue lysates. Multiplexing and flexible target selection enables comprehensive analysis of target proteins from a single sample.

MATERIALS AND METHODS

Cell Lines and Tissue Lysate:

Cell lines were obtained from American Type Culture Collection (ATCC) and Tumor Biology Collection (TBC, BioIVT, Thermo Fisher Scientific, San Jose, CA). Patient derived xenograft samples were lysed in 2% SDS prior to multiplex IP enrichment followed by Targeted MS (Pierce LC System). For patient derived xenograft samples, absolute quantitation was obtained from five patient derived lung tumor xenograft samples using the SureQuant™ Pathway kit. Patient derived xenograft samples were lysed in 2% SDS before multiplex IP enrichment followed by Targeted MS (Pierce LC System). For patient derived xenograft samples, absolute quantitation was obtained from five patient derived lung tumor xenograft samples using the SureQuant™ Pathway kit.

Methods:

Multiplex immunoprecipitation (IP) was performed using the Thermo Scientific Multiplex IP Lysis buffer (PN#87788) supplemented with 1X IP Lysis buffer. The samples were incubated for 1 hour at 4°C and then centrifuged at 10,000 x g for 5 minutes. The supernatant was removed and the lysate was stored at -80°C for subsequent analysis. Multiplex immunoprecipitation was performed using the Thermo Scientific Multiplex IP Lysis buffer (PN#87788) supplemented with 1X IP Lysis buffer. The samples were incubated for 1 hour at 4°C and then centrifuged at 10,000 x g for 5 minutes. The supernatant was removed and the lysate was stored at -80°C for subsequent analysis.

RESULTS

The development of multiplex IP assays enabled comprehensive analysis of target proteins from a single sample.

CONCLUSIONS

Multiplexing and flexible target selection enable comprehensive analysis of target proteins from a single sample. The SureQuant™ Pathway panels are amenable to diverse sample sources and allowed identification of target proteins from cellular and tissue lysates.