Bead Assisted Mass Spectrometry (BAMSTM): A Robust Affinity Capture, MS Method for Multiplexed Biomarker Profiling

Sergey Mamaev1, Jeffrey C. Silva1, Camilla Worsfold2, Matthew P. Stokes2, Kimberly A. Lee2, Morty Razavi3, N. Leigh Anderson3 & Vladislav B. Berge2

1Adeptrix Corporation, Beverly, MA 01915, 2Cell Signaling Technology, Danvers MA 01923, 3iSCAPA Technologies, Victoria, British Columbia

INTRODUCTION

Proteomic studies that monitor protein and PTM abundances often employ multi-dimensional liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) for identification and quantification. However, one limitation of this method is that each protein or PTM is analyzed individually, which results in a limited number of analytes that can be interrogated. The BAMS assay is a high-throughput technology that simultaneously monitors specified analytes (targets, epitopes) and is capable of measuring target abundance with high sensitivity and specificity. Similar to bead-based assays, BAMS measures relative analyte abundance by capturing targets against a library of capture beads and monitoring the signal using MALDI-TOF mass spectrometry. The BAMS assay offers a unique advantage over other platforms by measuring and reporting both endogenous and exogenous analyte concentrations, enabling the detection of even rare analytes with a higher signal-to-noise ratio.

RESULTS

Figure 1. Targeted Proteins in Human BAMS Assay in HEP-2 Cells. (A) Analyte enrichment. (B) Sample filtration. (C) Protein expression. (D) SILAC Labeling

Figure 2. Targeted analytes. (A) MKN-45 and (B) 200 fg/µL nanomolar concentration of the PTM peptide was used in the front panel to assess the minimal analyte concentration of the assay to be captured efficiently. For protein and PTM biomarkers, each assay was combined using sensitivity and specificity. A minimum of 40 pg of analyte can be detected.

Figure 3. Multiple Modes of Quantitation for BAMS Assay. (A) Positive Standard - Basic Labile Protein. (B) Internal Standard - Lighter Free Spectrally. (C) Endogenous + labeled peptide (200 fmoles) - Light-heavy SILAC pairs for a subset of the 50-plex BAMS assay along with observed abundance of the target detected. (D) The BAMS assay was configured to efficiently monitor close to 75 protein biomarkers.

Figure 4. Rapamycin Treatment of Gastric Carcinoma. (A) Western ECL blotting substrate (BioRad). (B) Protein lysates and digested peptides. (C) Sample-1 (+85) Epitope. (D) Sample-2 Endogenous. (E) Sample-3 Endogenous + labeled peptide (1000 fmoles) + labeled peptide (200 fmoles) - Light-heavy SILAC pairs for a subset of the 50-plex BAMS assay along with observed abundance of the target detected.

Figure 5. Rapamycin Treatment of Gastric Carcinoma. (A) Western ECL blotting substrate (BioRad). (B) Protein lysates and digested peptides. (C) Sample-1 (+85) Epitope. (D) Sample-2 Endogenous. (E) Sample-3 Endogenous + labeled peptide (1000 fmoles) + labeled peptide (200 fmoles) - Light-heavy SILAC pairs for a subset of the 50-plex BAMS assay along with observed abundance of the target detected.

CONCLUSIONS

• BAMS assays have been developed to efficiently monitor close to 75 protein biomarkers from a variety of biological samples.
• The binding capacity of a single bead is sufficient to measure the abundance of a target protein in a sample.
• BAMS assays have been configured to efficiently monitor close to 75 protein biomarkers.
• The BAMS assay was configured to efficiently monitor close to 75 protein biomarkers.

REFERENCES


4. Adeptrix Corporation, Beverly, MA 01915, Cell Signaling Technology, Danvers MA 01923, iSCAPA Technologies, Victoria, British Columbia