Comparative Performance Evaluation of the Mass Spectrometric Immunoassay (MSIA™) and Magnetic Bead Formats

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Application Notes MSIA1003

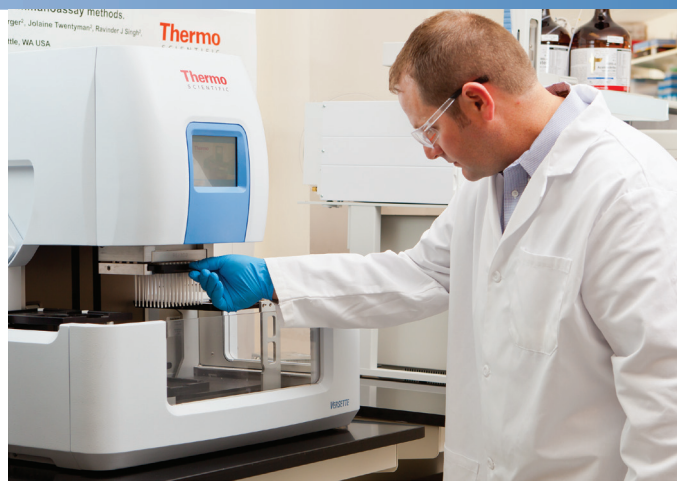
Key Words

Mass Spectrometric Immunoassay, Protein A/G, MSIA-Tips, Magnetic Beads, Background, Limit of Detection/Limit of Quantification, Insulin-Like Growth Factor 1 (IGF1)

Introduction

The use of Mass Spectrometry (MS) in protein/peptide detection has become the staple in proteomics applications. The analysis of selected proteins from complex biological fluids is readily achievable using LC/MS/MS methods. These methods have repeatedly demonstrated the ability to detect thousands of proteins/peptides from a single sample; however, the complexity of these samples has made routine and consistent analysis troublesome. In order to overcome these issues, the introduction of immunoaffinity capture and enrichment as a part of the front-end processing has become widely adopted. Now known as the Mass Spectrometric Immunoassay (MSIA)[1-4], this specific target enrichment procedure provides a cleaner and more consistent sample for analysis, and in addition, allows for the detection of much lower abundant proteins not previously achievable in proteomics applications. It is therefore advantageous to have a highly efficient, reproducible, and effective technology to perform such an enrichment step.

Presented here is a description of a fast and effective approach to performing immunoaffinity sample purification. Using a novel immunoaffinity enrichment technology called the Thermo Scientific™ Protein A/G MSIA™-Tips, a head-to-head comparison against traditional Protein A/G magnetic beads was performed. In this study we specifically examined the lower limits of detection and quantification (LLOD and LLOQ, respectively), as well as non-specific binding of both analyte extraction formats using an Insulin-Like Growth Factor 1 (IGF1)[5-6] model system (see Technical Note: MSIA1001). This testing demonstrated that the MSIA-Tips provide a 10-fold improvement in the LLOD and a 20-fold improvement in the LLOQ over a comparable volume of beads. The MSIA-Tips also provided higher quality immuno-affinity purification that resulted in less non-specific binding. This resulted in a 55% increase in IGF1 percent selectivity over the beads tested.



Experimental

Materials

- Thermo Scientific Protein A/G MSIA-Tips
- Thermo Scientific Versette Liquid Handling Platform
- BcMag™ Protein A/G Magnetic Beads (Bioclone Inc.)
- Magnetic Stand
- Anti-human IGF1 antibody
- Human recombinant IGF1 (IGF1 standard)
- Recombinant LR3-IGF1 (Internal reference standard)
- Human EDTA Plasma
- Thermo Scientific TSQ Vantage™ Triple Stage Quadrupole Mass Spectrometer
- Thermo Scientific LTQ Orbitrap™ XL
- Thermo Scientific Hypersil GOLD™ C18 column (50 mm x 2.1 mm, 1.9 µm particle size)

Thermo SCIENTIFIC

Method

Antibody Loading

The BcMag™ Protein A/G Magnetic Beads and the Protein A/G MSIA-Tips utilized the same affinity reagents in this study. Both were loaded with anti-IGF1 antibody using the recommended manufactures' protocols. In order to provide more consistent analyses, a magnetic stand was used in the bead applications while the Versette platform was used with the tips.

Extraction and Enrichment

Once loaded, both formats were ready for sample interrogation. However, in order to perform a representative head-to-head comparison, the volume of beads used was scaled in order to match the fixed volume of the micro-columns within the MSIA-Tips prior to application. The bead volume required was 0.6 uL, which was calculated based on the published binding capacity values for both technologies.

The samples used, calibrants (1.0, 2.5, 5.0, 10 and 20 ug/L) and plasma samples, were prepared following the protocol provided in Technical Note: MSIA1001. In these experiments, the calibrants were analyzed in triplicate (n = 3) and used for the determination of both the LLOD and the LLOQ. The human plasmas were used for the background assessment and were performed in decaplet (n = 10). The beads were applied following the manufacturer's suggested protocols and the Protein A/G MSIA-Tips were used as described in Technical Note: MSIA1001. Post incubation, both formats were subject to a series of rinse steps followed by elution. The same rinse and elution buffers were used with each. The extracted proteins using both methods were then subjected to identical post extraction processing (i.e., reduction, alkylation, trypsinization, etc.). These processing protocols can also be found in Technical Note: MSIA1001.

Analysis

In this study, two forms of LC-MS/MS analyzers were used:

1) Thermo Scientific LTQ Orbitrap XL – for the assessment of format non-specific binding,

2) Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer – used for SRM analysis in the determination of the LLOD and LLOQ of each affinity format.

Both MS/MS systems utilized a Thermo Scientific Accela™ pump, a CTC PAL® autosampler, and a Thermo Scientific Hypersil GOLD C18AQ fused silica capillary column for the LC systems. LC-MS/MS runs were performed using standard protocols for the analysis of the IGF1 tryptic peptides of interest (originating from IRS and human), as described in Technical Note: MSIA1001.

Results and Discussion

Assessment of Non-Specific Binding

Data for the assessment of non-specific background interference was obtained using both LC-MS/MS systems. Figure A shows representative Mass Spectra observed from the Protein A/G MSIA-Tips and the Protein A/G BcMag Magnetic Beads extracted samples using the LTQ Orbitrap XL. The MSIA-Tips were able to more efficiently extract the IGF1 from the samples than compared to the beads. As shown in (Figure 1: Top), there was far less background using the tips than observed with the bead method (Figure 1: Bottom). The data presented showed a 55% improvement in the IGF1 signal vs. the detected background. This decrease in non-specific binding provides a clear analytical benefit as the improved percent selectivity will translate into improved detection and signal stability in SRM analyses.

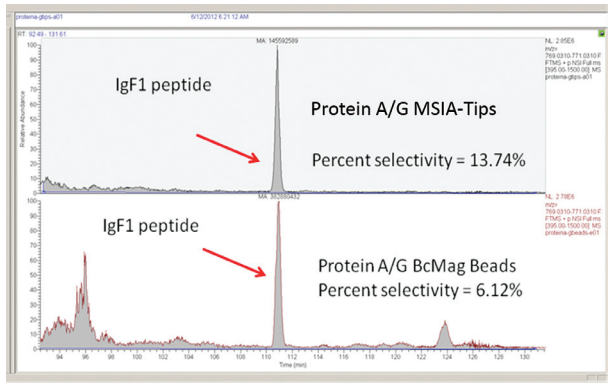


Figure 1. Resultant LTQ Orbitrap XL MS/MS data of IGF1 obtained from both the MSIA-Tips (Top panel) and BcMag™ Magnetic Bead (Bottom panel) protein capture and enrichment. Data clearly shows less interferences for the Tip extraction vs. the Bead when detecting the peptide of interest.

When the SRM analysis was performed on the same peptides using the TSQ Vantage, the data supported the previously discussed benefits. This is shown in Figure 2, in which the representative chromatograms of the SRM analyses illustrate the influence of these non-specifically retained contaminants. The observed chromatograms from the bead extractions showed large inconsistencies between the two extraction formats within the MS window of the IGF1 target peptide. Moreover, the problem is compounded by the presence of additional contaminant peaks, as both issues limited the sensitivity

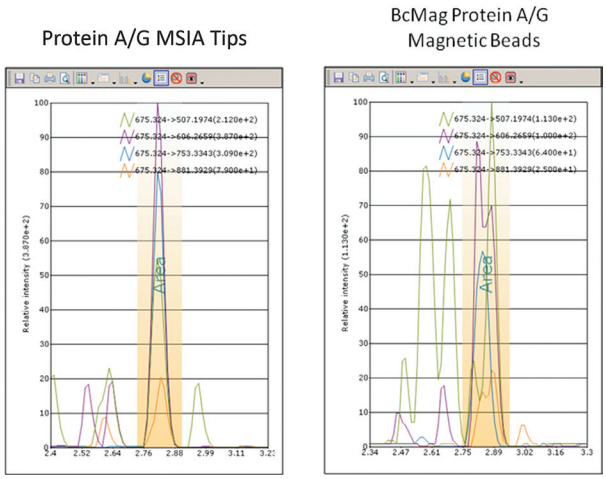


Figure 2. Representative data generated using the TSQ Vantage of the same IGF1 peptide. The tip extraction method resulted in more uniform peak shape and less interference within the elution window than with the beads.

and the accuracy of the assay.

This sensitivity was assessed using SRM analysis, in which the LLOD and the LLOQ of both assay extraction formats were determined. These characteristics were determined by evaluating the standard deviations of the sample replicates as each of the calibration points. An observed overlap of ≥ 50% between successive calibration points was used as a threshold for establishing both points. Using these metrics, data clearly showed a ≥ 10-fold improvement in both the LLOD and the LLOQ using the Tips vs. the Bead format. This data is presented in Table A.

Extraction Format	Manufacturer	LLOD (ng/mL) (n = 3)	LLOQ (ng/mL) (n = 3)
MSIA-Tips	Thermo Fisher Scientific	1	1
BcMag™ Magnetic Beads	Bioclone Inc.	10	20

Table A. The established LLOD and LLOQ of both the Protein A/G MSIA-Tips and the BcMag™ Magnetic Bead immunoaffinity extraction systems.

Conclusion

The Protein A/G MSIA-Tips demonstrated the ability to generate enhanced results over the BioClone Protein A/G BcMag™ Beads. Not only were the MSIA-Tips able to demonstrate superior LLOD and LLOQ using a standard tryptic digest LC-MS/MS SRM workflow over a comparable capacity of beads, but more specific signal due to decreased non-specific binding. Both these benefits were directly associated to one another, and these results clearly demonstrated such technical benefits which instill confidence in the data generated by the end user. Other characteristics of this model system using the Protein A/G MSIA-Tips were established and can be found in Technical Note: MSIA1002

References

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Ordering Information

MSIA D.A.R.T'S Pipette Tips		
Compatible with the Thermo Scientific Versette Automated Liquid Handler, Thermo Scientific Finnpipette® Novus i Multichannel Electronic Pipettes (for immuno-precipitation), also with select Eppendorf®, Biohit® and Hamilton® Multichannel Pipettes.		
Cat. No.	**Description**	**Packaging**
991PRT11	300 µl MSIA D.A.R.T.'S, Protein A	Pack of 96 tips
991PRT12	300 µl MSIA D.A.R.T.'S, Protein A	Pack of 24 tips
991PRT13	300 µl MSIA D.A.R.T.'S, Protein G	Pack of 96 tips
991PRT14	300 µl MSIA D.A.R.T.'S, Protein G	Pack of 24 tips
991PRT15	300 µl MSIA D.A.R.T.'S, Protein A/G	Pack of 96 tips
991PRT16	300 µl MSIA D.A.R.T.'S, Protein A/G	Pack of 24 tips
991CUS02	300 µl MSIA D.A.R.T.'S, Custom*	Pack of 96 tips
991R	300 µl MSIA D.A.R.T.'S, Reloadable Rack	1 reloadable rack, tips are not included

Automated Liquid Handling Platform and Pipetting Head	
Cat. No.	**Description**
650-01-BS	Versette Base Unit Stage, Head Housing and Pipetting Head Required for Use
650-02-NTC	96- and 384-Channel Housing Assembly. For Use with 96- and 384-Channel Pipetting Heads
650-03-SPS	6-Position Stage, Guarding Included.
650-06-96300	96-Channel Air Displacement Pipetting Head. Volume 5-300 µl
650-04-PUMP	Pump Module Optional Accessory, Used for Tip Washing/Reagent Replenishing
650-05-96TTW	96-Channel Tip Wash Station, Tall, Optional Accessory
650-08-96300SD	Serial Dilute Magazine 96/300 µl (8/12)

Multichannel Pipettes and Pipette Stand		
Cat. No.	**Description**	**Quantity**
46302000	Thermo Scientific Finnpipette® Novus i Electronic 8-Channel Pipette, 20-300 µl (for immuno-precipitation)	1 pipette
46302100	Finnpipette Novus i Electronic 12-Channel Pipette, 20-300 µl (for immuno-precipitation)	1 pipette
991S	Finnpipette Novus i Adjustable Pipette Stand (for immuno-precipitation)	1 pipette stand
991SP8	Finnpipette Novus i Electronic 8-Channel Pipette, 20-300 µl and Pipette Stand (for immuno-precipitation)	1 pipette and 1 pipette stand
991SP12	Finnpipette Novus i Electronic 12-Channel Pipette, 20-300 µl and Pipette Stand (for immuno-precipitation)	1 pipette and 1 pipette stand

Liquid Chromatography
Description
Thermo Scientific Dionex™ UltiMate® 3000 RSLCnano Systems
Thermo Scientific Hypersil GOLD™ C18 column (50 mm x 2.1 mm, 1.9 µm particle size)

Mass Spectrometry and Software
Description
Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer
Thermo Scientific Pinpoint Software
Thermo Scientific Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer
Thermo Scientific LTQ Orbitrap XL

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