

Demonstration of Performance Characteristics of Protein A, G and A/G MSIA™-Tips

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Key Words

Mass Spectrometric Immunoassay, MSIA-Tips, Protein A, Protein G, Protein A/G, Protein Quantification, Immunoaffinity, LC-MS/MS, Insulin-Like Growth Factor 1 (IGF1)

Introduction

The Mass Spectrometric Immunoassay (MSIA)¹ is a common analytical method that combines highly sensitive MS detection with upfront immuno-affinity sample purification²⁻⁴. This hybridized approach provides the ability to analyze much lower abundant proteins in a given proteome than previously achieved. However, as the mainstay in proteomics applications; more robust, consistent, and versatile methods for performing this sample purification and enrichment step are a growing necessity.

Presented here are the results of a study that tested a novel technology that addresses these sample preparation needs. Tested were the various MSIA-Tips (Protein A, G and A/G), an immuno-affinity extraction technology in a pipette tip format that can be customized by the end-user to target a specific analyte of interest. Replicate calibration curves and plasma sample analyses were performed on all three surfaces using an Insulin-Like Growth Factor 1 (IGF1)⁵⁻⁶ model system (see Technical Note: MSIA1001). These results demonstrate the stability and consistency of the MSIA-Tip performance, in which all three tip types universally displayed large dynamic ranges (1 – 1500 ng/mL), protein recovery of > 85%, and coefficients of variation ≤ 10%.



Materials

- Thermo Scientific™ Protein A, G and A/G MSIA-Tips
- Thermo Scientific Versette Liquid Handling Platform
- Thermo Scientific Finnpiptette® F1 Adjustable-Volume Pipettes
- 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 Hypersil GOLD™ C18 column (50mm x 2.1mm, 1.9 µm particle size)

Method

The workflow for the use of Protein A, Protein G, and Protein A/G MSIA-Tips is demonstrated in Technical Note: MSIA1001.

Samples

Samples were prepared as described in Technical Note: MSIA1001. An 8-point IGF1 calibration curve consisting of 1, 5, 10, 25, 100, 500, 1000, and 1500 ng/mL of IGF1 was prepared in triplicate. Samples from a single EDTA plasma donor were prepared in replicate ($n = 12$) and used to evaluate reproducibility, while plasma samples spiked with increasing amounts of IGF1 (100 ng/mL, 200 ng/mL, and 400 ng/mL) were used to establish protein recovery.

Antibody Loading of MSIA-Tips

The various MSIA-Tips (Protein A, Protein G and Protein A/G) were loaded with 100 μ L rabbit anti-human IGF1 antibody (0.01 mg/mL) following protocols provided in the user manuals (total processing time 30 minutes).

IGF1 Extraction and Enrichment

Co-extraction and enrichment of IGF1 and LR3-IGF1 were performed using a single MSIA-Tip (loaded with antibody) per each sample following the protocols provided in the user manuals (total processing time 30 minutes). After extraction, captured protein was eluted and processed for SRM which were performed on TSQ Vantage Triple Quadrupole Mass Spectrometer as described in Technical Note: MSIA1001.

Results and Discussion

The performance characteristics of the MSIA-Tips (Protein A, Protein G, and Protein A/G) were tested using a model system MSIA-SRM, based on IGF1 (see Technical Note: MSIA1001). IGF1 was extracted and enriched from plasma and diluted IGF1 samples using each of the MSIA-Tips. Performance characteristics of lower limit-of-detection (LLOD), assay dynamic range (limits-of-quantification, LOQ), reproducibility, and protein recovery were all evaluated.

The IGF1 calibration curves generated, using each of the MSIA-Tips, are shown in Figures 1A, 1B and 1C. As shown, linear responses over a wide dynamic range (1 to 1500 ng/mL of IGF1) were exhibited by each of the MSIA D.A.R.T.'S. The tips offered limits-of-detection as low as 5.2 femtomole of IGF1 from diluted samples (40 μ L sample volume at 1 ng/mL) as shown in Table A, with no additional sample preparation or protein depletion. These findings are a result of the tips' capacities and their chemically inert designed solid support, which provides high signal-to-noise with significantly reduced background (see Technical Note: MSIA1003). Extraction efficiency also contributes to these performance characteristics, and as observed, the protein recovery using these devices is greater than 85% (Table B). Furthermore, the tips were found to provide significant reproducibility. From the analyses of replicate plasma samples, which endogenous IGF1 was extracted and enriched, CVs of 10% and less (Table C) were exhibited. This results in significant user confidence in using these devices.

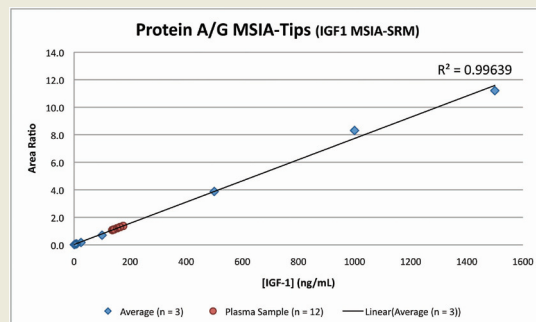


Figure 1A. An 8-point IGF1 calibration curve was generated from the extraction and enrichment of IGF1 from a dilution series of IGF1 standard using Protein A/G MSIA-Tips. The MSIA-SRM measurement of IGF1 from replicate plasma samples ($n = 12$) resulted in an 8.5% CV.

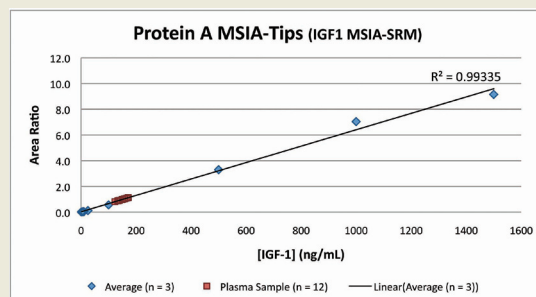


Figure 1B. An 8-point IGF1 calibration curve was generated from the extraction and enrichment of IGF1 from a dilution series of IGF1 standard using Protein A MSIA-Tips. The MSIA-SRM measurement of IGF1 from replicate plasma samples ($n = 12$) resulted in a 10% CV.

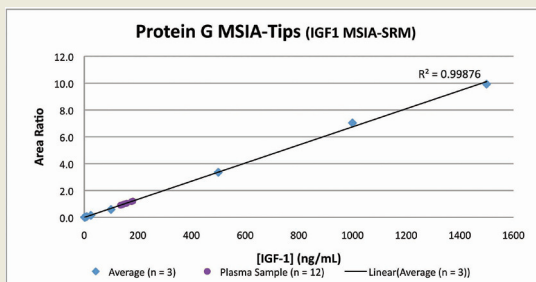


Figure 1C. An 8-point IGF1 calibration curve was generated from the extraction and enrichment of IGF1 from a dilution series of IGF1 standard using Protein G MSIA-Tips. The MSIA-SRM measurement of IGF1 from replicate plasma samples ($n = 12$) resulted in a 8.6% CV.

Figure 1. 8-point IGF1 calibration curves were generated from standard samples consisting of 1, 5, 10, 25, 100, 500, 1000, and 1500 ng/mL of IGF1 and 500 ng/mL of the internal standard (LR3-IGF1). Using Protein A, Protein G, and Protein A/G MSIA-Tips, reproducible target enrichment down to low femtomoles, while making quantification measurements possible over a wide linear dynamic range were observed.

MSIA-Tip Type	Protein A	Protein G	Protein A/G
LLOD	1 ng/mL (5.2 femtomole)	1 ng/mL (5.2 femtomole)	1 ng/mL (5.2 femtomole)
LOQ	10 - 1500 ng/mL (52 - 7800 femtomole)*	1 - 1500 ng/mL (5.2 - 7800 femtomole)*	1 - 1500 ng/mL (5.2 - 7800 femtomole)*

* Amounts based on a 40 µL plasma sample volume.

Table A. Protein A, G and A/G MSIA-Tips enable femtomole detection of targeted analytes, with significantly large dynamic ranges for quantification reducing the need for repeats on samples with high IGF1 levels.

MSIA-Tip Type	Protein A	Protein G	Protein A/G
% Recovery (average, n = 3)	104 %	85 %	114 %

Table B. Protein A, G and A/G MSIA-Tips demonstrate highly efficient recoveries from biological samples without any additional sample preparation or protein depletion.

MSIA-Tip Type	Protein A (n=12)	Protein G (n=12)	Protein A/G (n=12)
Reproducibility (%CV)	10 %	8.6 %	8.5 %

Table C. Protein A, G and A/G MSIA-Tips exhibit exceedingly reproducible quantification of IGF1, providing users with confidence in their performance.



Conclusion

We clearly demonstrated in this study that the Protein A, Protein G, and Protein A/G MSIA-Tips are able to perform highly reproducible and effective front end immunoaffinity sample enrichment for subsequent MS detection and SRM quantification. Using this specific IGF1 model system, the MSIA-Tips demonstrated highly consistent assay performance and characteristics that provide the end user with a high degree of confidence in their results. This Protein A/G based MSIA-Tip format also provide the user with the flexibility to tailor these devices for use with their own specific antibodies and applications. As demonstrated in these results, the functional pipette tip format of these devices is able to address large sample cohorts using a Versette liquid handler. Regardless of the affinity ligand (Protein A, G or A/G) used, the MSIA-Tips generated uniform results using our IGF1 model system (see Technical Note: MSIA1001). Additional characteristics of the Protein A/G based MSIA-Tips, including background and a head-to-head comparison with protein A/G magnetic beads, can be found in Technical Note: MSIA1003.

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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 Finnpiptette® 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 Finnpiptette Novus i Electronic 8-Channel Pipette, 20-300 µl (for immuno-precipitation)	1 pipette
46302100	Finnpiptette Novus i Electronic 12-Channel Pipette, 20-300 µl (for immuno-precipitation)	1 pipette
991S	Finnpiptette Novus i Adjustable Pipette Stand (for immuno-precipitation)	1 pipette stand
991SP8	Finnpiptette Novus i Electronic 8-Channel Pipette, 20-300 µl and Pipette Stand (for immuno-precipitation)	1 pipette and 1 pipette stand
991SP12	Finnpiptette 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		

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