A Universal Mass Spectrometric Immunoassay (MSIA™) Model System Based on Human Insulin-Like Growth Factor 1 (IGF1)

Eric E. Niederkofler, Senior Scientist, David A. Phillips, Technician, Dobrin Nedelkov, Manager of Research and Development and Urban A. Kiernan, Senior Scientist, Thermo Fisher Scientific, Tempe, Arizona.

Bryan Krastins, Senior Applications Scientist, Scott Peterman, Senior Applications Scientist, Alejandra Garces, Senior Applications Scientist and Mary F. Lopez, BRIMS Center Director, BRIMS Center, Thermo Fisher Scientific, Cambridge, Massachusetts.

Key Words

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

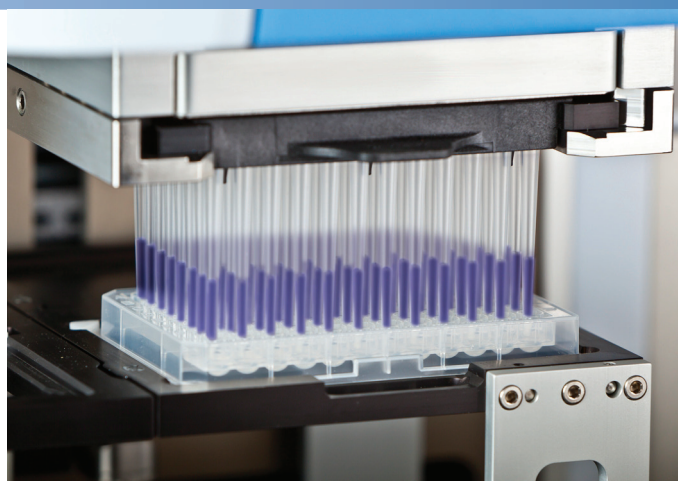
Introduction

Immunoaffinity capture and enrichment of protein and peptide targets is a widely utilized method for sample cleanup prior to mass spectrometric analysis. Termed the Mass Spectrometric Immunoassay (MSIA)[1], this hybridized combination of micro-scale purification with highly sensitive detection has repeatedly demonstrated the ability to detect much lower abundance proteins from a given proteome as compared to other fractionation methods[2,3]. Even though this approach to sample purification is now the staple in proteomics methods; more robust, consistent, and versatile methods for performing this sample purification and enrichment step are a growing necessity.

To further improve upon this front-end sample processing, a new technology, Thermo Scientific™ MSIA™, was tested. In the form of a functional pipettor tip, their performance was evaluated (see Application Notes: MSIA1002 and MSIA1003) and bench marked against other technologies. However, to perform these test, a model system translatable to various ligand surfaces including Protein A, Protein G and Protein A/G was needed. Presented are the results of a developed model system for the Protein A/G MSIA-Tips based on Insulin-Like Growth Factor 1(IGF1), a clinically relevant endocrine and oncology protein biomarker [4,5,6].

Materials

- Thermo Scientific Protein A/G MSIA-Tips
- Thermo Scientific Versette Liquid Handling Platform
- Anti-human IGF1 antibody
- Human recombinant IGF1 (IGF1 standard)
- Recombinant LR3-IGF1 (Internal reference standard)
- Thermo Scientific Pierce BupH Phosphate Buffered Saline (PBS)



- Antibody dilution buffer - 10mM MES/0.1% polysorbate 20, pH 5
- EDTA plasma, human donor
- Trypsin
- LC-MS grade water
- Thermo Scientific Optima grade Formic Acid (FA)
- Thermo Scientific Optima grade Acetonitrile (ACN)
- Standards dilution buffer - 10 g/L BSA in PBS pH 7.2
- Sample dilution buffer - PBS/0.3% SDS
- Elution buffer - 33% acetonitrile/0.4% trifluoroacetic acid
- Reduction buffer - 10mM DTT in 30% isoproponal/0.1M ammonium bicarbonate pH 8.0
- Alkylation reagent - 0.5M Iodoacetamide/0.1M ammonium bicarbonate pH 8.5
- Thermo Scientific TSQ Vantage™ Triple Stage Quadrupole Mass Spectrometer
- Thermo Scientific Hypersil GOLD™ C18 column (50 mm x 2.1 mm, 1.9 µm particle size)

Thermo SCIENTIFIC

Methods

Samples

An 8-point IGF1 calibration curve was prepared by serial dilutions of recombinant human IGF1 into standards dilution buffer (concentration range 1-1500 ng/mL IGF1). Replicate samples (n = 12) from a single EDTA plasma donor and IGF1 calibration curve samples were prepared by diluting 40 µL plasma/IGF1 standards with 20 µL 0.5 mg/L internal reference standard (in standards dilution buffer) followed by 100 µL PBS/0.3%SDS and incubated at room temperature for 30 minutes prior to extraction and enrichment with Protein A/G MSIA-Tips.

Antibody Loading of Protein A/G MSIA-Tips

Protein A/G MSIA-Tips were loaded with 100 µL rabbit anti-human IGF1 antibody (0.01 mg/mL) following protocols provided in the user manual (total processing time 30 minutes).

IGF1 Extraction and Enrichment

Co-extraction and enrichment of IGF1 and LR3-IGF1 were performed using a single Protein A/G MSIA-Tip (loaded with antibody) per each sample following the protocols provided in the user manual (total processing time 30 minutes). After extraction, IGF1 and LR3-IGF1 were digested and analyzed by SRM as described below.

Sample Elution and Trypsin Digestion

Captured IGF1 and LR3-IGF1 were co-eluted from the Protein A/G MSIA-Tips by repetitively mixing (20 cycles of aspirating and dispensing 30 µL volumes) 50 µL of elution buffer within the wells of a 96-well plate using the MSIA-Tips with captured IGF1 and LR3-IGF1, thus eluting IGF1 and LR3-IGF1 into the 50 µL elution buffer within each well. Samples were lyophilized to dryness and then re-suspended in 30 µL of reduction buffer and allowed to reduce for 30 minutes at 37°C. Reduced samples were then alkylated by adding 2.4 µL alkylation reagent and incubating in the dark at room temperature for 30 minutes. Reduced and alkylated samples were diluted with 92.5 µL of warm (50°C) 0.1M NH_4HCO_3/ 5mM $CaCl_2$ and then digested by adding 25 µL 4 mg/L trypsin to each sample. Samples were allowed to digest for 2 hours at 50°C and then stopped by adding 5.3 µL of acid solution (3 µL 100% formic acid and 2.3 µL 1mg/mL glucagon). Injection volumes of 155 µL of the digests were injected into the LC-MS for SRM.

SRM Methods

SRM methods were developed on a Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer with a Thermo Scientific Accela™ pump, a CTC PAL® auto-sampler, and a Thermo Scientific Ion Max source equipped with a high-flow metal needle. A mass window of full width at half maximum of 0.7 (unit resolution) was used in the SRM assays because immuno-enriched samples had a very high signal-to-noise ratios. Reversed-phase separations were carried out on a Hypersil GOLD™ C18 column (50 mm x 2.1 mm, 1.9 µm particle size) with a flow rate of 240 µL/minute. Solvent A was 0.2% formic acid, and solvent B was 0.2% formic acid in acetonitrile.

Results and Discussion

A model system for the Protein A/G MSIA-Tips, based on Insulin-Like Growth Factor 1(IGF1), was developed to serve as a template for future LC-MS/MS methods that perform quantitative immuno-affinity proteomics. The Protein A/G MSIA-Tip workflow (Figure 1) proved to be simple and fast, requiring as little as 1 hour to provide immuno-purified IGF1. Using the Versette liquid handler equipped with these tips, up to 96 samples were able to be processed in parallel with minimal user interaction.

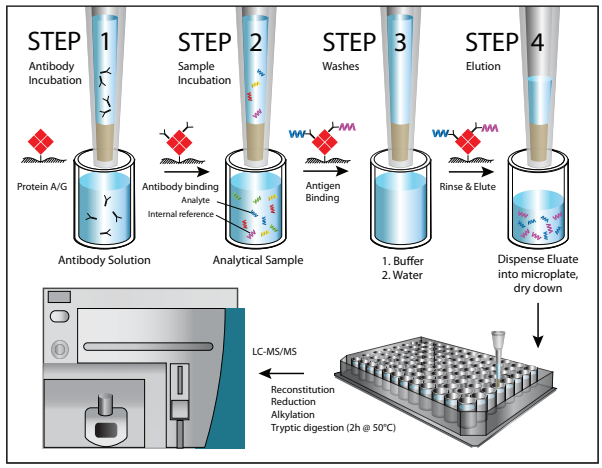


Figure 1. Protein A/G MSIA-Tip Workflow: Protein A/G MSIA-Tips are used by repetitive pipetting of solutions, allowing for efficient interaction between capture and analyte reagents within the porous monolithic solid supports. First, antibody is loaded onto the Protein A/G surface. The loaded antibody is then used to immuno-capture and enrich its targeted analyte from biological samples. After rinsing, retained analyte is eluted and subjected to reduction, alkylation and standard tryptic digestion and LC-MS/MS protocols.

The resulting IGF1 MSIA-SRM, using Protein A/G MSIA-Tips, demonstrated a wide linear dynamic range (Figure 2) and the ability to reproducibly quantify unknown IGF1 concentrations from plasma samples (Table A). Loaded with IGF1 antibody, the Protein A/G MSIA-Tips enabled for the efficient capture and enrichment of femtomole amounts IGF1 (Table A) with no additional sample preparation or depletion.

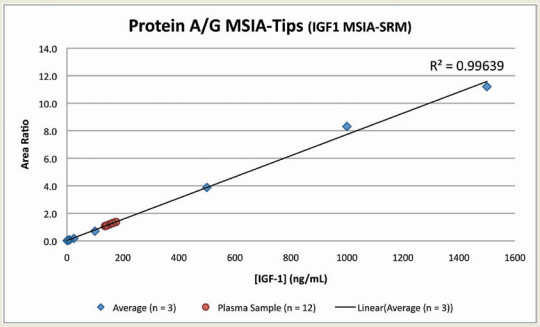


Figure 2. An 8-point IGF1 calibration curve was generated from standard samples consisting of 1, 5, 10, 25, 100, 500, 1000, and 1500 ng/mL of IGF1 and 500 ng/mL of the internal standard (LR3-IGF1). MS area ratios of IGF1:LR3-IGF1 are plotted against the IGF1 concentrations and used to calculate the concentrations of IGF1 in unknown samples based on the samples' area ratios. Replicate analyses of a single plasma donor demonstrated CV of 8.5%.

LLOD	Assay Range	IGF1 Recovery	Reproducibility, %CV (n = 12)
1 ng/mL (5.2 femtomole)*	1 - 1500 ng/mL (5.2 - 7800 femtomole)*	114 %	8.5 %

*Amounts based on a 40µL plasma sample volume.

Table A. Protein A/G MSIA-Tips IGF1-SRM assay characteristics.

Conclusion

We described the development of a novel model system, targeting human IGF1, to test the performance of Protein A/G MSIA-Tips. Using this test system, the MSIA-Tips demonstrated the ability to reproducibly perform quantitative measurements using SRM detection. These results clearly show how these devices can provided a mechanism for the highly specific and reproducible enrichment of a target analyte from plasma. Not only does this described SRM assay provide a uniform approach to technology evaluation, it also serves as a template for the development of future LC-MS/MS based MSIA methods.

References

1. Nelson, R.W.; Krone, J.R.; Bieber, A.L.; Williams, P. Mass spectrometric immunoassay Anal. Chem. 1995, 67, 1153– 1158.

2. Niederkofler, E.E.; Kiernan, U.A.; O'Rear, J.; et al. Detection of Endogenous B-Type Natriuretic Peptide at Very Low Concentrations in Patients With Heart Failure. Cir. Heart fail. 2008, 4,258-264

3. Lopez, M.F.; Rezai, T.; Sarracino, D.A.; et al. Selected Reaction Monitoring-Mass Spectrometric Immunoassay Responsive to Parathyroid Hormone and Related Variants. Clin. Chem. 2010, 56, 281-290.

4. Monzavi, R.; Cohen, P. IGFs and IGFBPs: role in health and disease. Best Pract. Res. Clin. Endocrinol. Metab. 2002, 16, 433-447.

5. Hankinson, S. E.; Willett, W. C.; Colditz, G. A., et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet. 1998, 351, 1393-1396.

6. Wolk, A.; Mantzoros, C. S.; Andersson, S. O., et al. Insulin-like growth factor 1 and prostate cancer risk: a population- based, case-control study. J. Natl. Cancer Inst. 1998, 90, 911-915.

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	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
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

thermoscientific.com/msia

Products are intended for research use only.

© 2012 Thermo Fisher Scientific Inc. All rights reserved. CTC PAL is a registered trademark of Leap Technologies. "Eppendorf" is a registered trademark of Eppendorf AG. "Biohit" is a trademark of Biohit Oy. "Hamilton" is a trademark of Hamilton Robotics.. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. Not all products are available in all countries. Please consult your local sales representative for details.

North America: +1 800 995 2787 • info.sandiego@thermofisher.com
Outside North America: +1 858 453 7551 • info.sandiego@thermofisher.com

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

APAAMSIAIGF10712