Thermo Scientific MSIA Streptavidin D.A.R.T.'S: Robust Immunoenrichment Process and Reproducibility Across Multiple Labs

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Key Words

Mass Spectrometric Immunoassay, MSIA, Streptavidin, Protein Quantification, Immunopurification, LC-MS/MS

Goal

To identify the reproducibility in the performance of Thermo Scientific™ MSIA™ Streptavidin D.A.R.T.'S® across multiple sites to enable efficient extraction and enrichment of a targeted protein from biological media.

Introduction

The Mass Spectrometric Immunoassay (MSIA), a legacy approach dating back to 1995,[1] is a hybridized technique that combines immunoaffinity protein analyte purification with high sensitivity mass spectrometric (MS) detection. By modifying the distal end of a pipette tip with a porous solid monolithic support, these devices can be derivatized with specific affinity ligands for the selective capture and enrichment of target analytes. This format has repeatedly demonstrated its superiority in the micro-scale immuno-affinity purification and enrichment of analytes for subsequent high sensitivity MS detection.[2,3,4] These pipette tip devices are now within the Thermo Scientific portfolio and are being developed into the next generation format that provides the end user with improved analytical performance, unparalleled versatility, stability, and ease of use.

Presented here is the use of MSIA Streptavidin D.A.R.T.'S for the immunoaffinity enrichment of a targeted protein, Insulin-Like Growth Factor[1] (IGF1).[5,6] We demonstrate the ability of these streptavidin-derivatized tips to efficiently and reproducibly (across multiple laboratories) enable the specific extraction and enrichment of a targeted protein from biological samples via a biotin-conjugated antibody complex.

Materials

- Thermo Scientific MSIA Streptavidin D.A.R.T.'S
- Thermo Scientific™ Finnpipette® Novus™ i Electronic 12-channel Pipette, 5 to 300µL
- Thermo Scientific™ Versette™ Automated Liquid Handler, equipped with 96-channel pipetting head
- Thermo Scientific™ Finnpipette*F1 Adjustable-volume Pipettes

- Biotin-conjugated 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
- Fisher Chemical Optima™ LC/MS Water
- Fisher Chemical Optima LC/MS Formic Acid (FA)
- Fisher Chemical Optima LC/MS 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% isopropanol/0.1M ammonium bicarbonate pH 8.0
- Alkylation reagent - 0.5M Iodoacetamide/0.1M ammonium bicarbonate pH 8.5
- Stop Solution - 40 mg/L ACTH1-24 (carrier peptide) in 57% Formic Acid
- Thermo Scientific™ TSQ-Vantage™
- Thermo Scientific™ Accucore™ aQ C18 column (50mm x 2.1mm, 1.9 mm particle size)

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Methods

To demonstrate the robust capabilities of the MSIA Streptavidin D.A.R.T.'S, five beta-test sites were selected to participate in a reproducibility study. Each test lab was equipped with MSIA Streptavidin D.A.R.T.'S, a Finnpipette Novus i Electronic 12-channel Pipette, and a MSIA immunoenrichment protocol. Following the provided protocol, the beta-test sites were instructed to load the provided biotin-conjugated anti-human IGF1 on to the MSIA Streptavidin D.A.R.T.'S, incubate the loaded antibody tips with the samples containing the target analyte (IGF1), and return the tips containing the enriched targeted analyte to the Thermo Fisher Scientific R&D Facility in Tempe, AZ for further processing and MS analysis following a protocol previously reported.[7]

Samples

Sets of IGF1 standard solutions consisting of a 6-point IGF1 calibration curve (25-1500 ng/mL), a multiple replicate (n=6) control (250 ng/mL), and a replicate (n=4) set of a high, medium, and low control (900, 600, 300 ng/mL), were prepared in parallel for 5 sites. Analytical samples were prepared by diluting an IGF1 standard solution (40 µL) with an internal reference standard solution (20 µL of 1 mg/L LR3-IGF1 in standards dilution buffer) followed by 0.3%SDS in PBS (100 µL). All samples were dried down and shipped to each of the five beta-test sites. Test sites were instructed to reconstitute each of the samples prior to extraction using the MSIA Streptavidin D.A.R.T.'S by adding water (160 µL) and allowing to stand for at least 15 minutes.

Loading of Streptavidin D.A.R.T.'S with biotin-conjugated antibody

MSIA Streptavidin D.A.R.T.'S were loaded with biotin-conjugated rabbit anti-human IGF1 antibody (100 µL of 0.03 mg/mL in 10mM phosphate buffered saline, PBS) by continuously aspirating and dispensing (1000 cycles, Speed Setting 1) the antibody solution (80 µL) with the Finnpipette Novus i Pipette. After 45 minutes of incubation, biotin-conjugated antibody was complexed to the immobilized streptavidin, resulting in functional antibody tips.

IGF1 Extraction and Enrichment

The functional antibody tips were used to co-extract and enrich IGF1 and LR3-IGF1 (internal reference standard). Immunoenrichment was performed by repetitively pipetting (100 cycles, 115 µL volume, Speed Setting 1) the samples using the Finnpipette Novus i Pipette. After the 7 minute extraction procedure, the MSIA Streptavidin D.A.R.T.'S were rinsed (100 cycles, Speed Setting 1) with PBS and then shipped back to the Tempe, AZ site for elution, post-processing, and LC-MS/MS analysis on a TSQ-Vantage.

Sample Elution and Trypsin Digestion

MSIA Streptavidin D.A.R.T.'S loaded with the immuno-enriched IGF1 and LR3-IGF1 were rinsed (10 cycles, 150 µL, Speed Setting 1) with water (200 µL) using a Finnpipette Novus i Pipette to wash away salts from previous rinses with PBS. This step was repeated a second time using fresh aliquots of water (200 µL) to ensure sufficient removal of salts. Elution buffer (100 µL) was added to wells of a 96-well PCR plate (AB1300) and captured IGF1 and LR3-IGF1 were co-eluted from the MSIA Streptavidin D.A.R.T.'S by repetitively mixing (20 cycles, 20 µL volumes, Speed Setting 1) the elution buffer. This method effectively eluted the IGF1 and LR3-IGF1 into the elution buffer within each well without disruption of the streptavidin/biotin complex. Samples were lyophilized to dryness and then re-suspended in reduction buffer (30 µL) for 30 minutes at 37° C. Reduced samples were then alkylated by adding alkylation reagent (2.4 µL) and incubated in the dark at room temperature for 30 minutes. Reduced and alkylated samples were diluted with warm 0.1M NH_4HCO_3/5mM $CaCl_2$ (92.5 µL at 50° C) and then digested by adding trypsin (25 µL of 4 mg/L trypsin) to each sample. Samples were allowed to digest for 2 hours at 50° C and then stopped by lowering the pH through the addition of 5.3 µL of Stop Solution. Injection volumes of the digests (155 µL) were injected into the LC-MS for SRM analyses.

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 (Leap Technologies), 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 immunoenriched samples had very high signal-to-noise ratios. Reversed-phase separations were carried out on an Thermo Scientific Accucore aQ HPLC column (100 mm x 2.1 mm, 2.6 µm particle size) with a flow rate of 240 µL/minute. Solvent A was Optima LC/MS 0.2% Formic Acid in Water and Solvent B was Optima LC/MS 0.2% Formic Acid in Acetonitrile.

Results and Discussion

A model system for the MSIA Streptavidin D.A.R.T.'S, based on Insulin-Like Growth Factor 1 (IGF1), was developed to serve as a template for future LC-MS/MS methods that perform quantitative immunoaffinity proteomics using biotin-conjugated antibodies or other affinity ligands. The MSIA workflow using MSIA Streptavidin D.A.R.T.'S (Figure 1) proved to be simple and fast, provide immunopurified targeted antigen, IGF1, in less than 90 minutes.

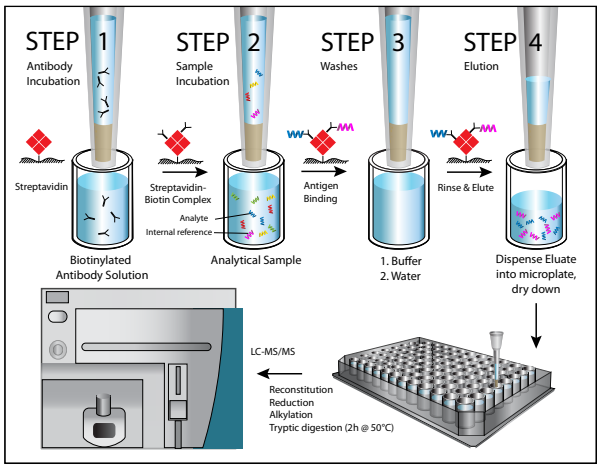

Figure 1. MSIA Streptavidin D.A.R.T.'S are used by repetitive pipetting of solutions, allowing for efficient interaction between capture and analyte reagents within the porous monolithic solid supports. First, a biotin-conjugated affinity ligand (i.e., antibody) is loaded onto the streptavidin surface. Loaded with the affinity ligand, MSIA Streptavidin D.A.R.T.'S are then used to immuno-capture and enrich the targeted analyte from samples. After rinsing, retained analyte is eluted from the affinity ligand without disruption of the biotin/streptavidin complex. The resulting purified and enriched analyte is then analyzed using standard LC-MS/MS methodologies.

Performance of the MSIA Streptavidin D.A.R.T.'S across and within multiple sites was evaluated, through the use of a biotin-conjugated anti-human IGF1antibody and the developed IGF1 MSIA SRM.[7] Table 1 summarizes the results of these evaluations. Within sites, individual labs demonstrated that MSIA Streptavidin D.A.R.T.'S were capable of reproducibly measuring the concentrations of the control samples, indicating the simplicity and consistent performance of the tips in multiple users' hands. This is further reinforced when comparing inter-site coefficients of variations, which provided CV's of less than 3%. This results in significant user confidence in the performance and use of these devices.

Beta Site	Reproducibility (CVs)	Accuracy
Site A	2 - 17%	93 - 109%
Site B	6 - 15%	95 - 106%
Site C	3 - 9%	99 - 101%
Site D	4 - 7%	99 - 101%
All Sites	2 - 3 %	97 - 103%

Table 1. MSIA Streptavidin D.A.R.T.'S provide a simple and reproducible method for the immunoenrichment of targeted analytes from complex biological matrices. This enables multiple users to obtain similar results, as demonstrated by the 2-3% CVs and the high accuracies (97-103%) for measuring the control samples across multiple sites.

Also evaluated was the accuracy of the five beta-test sites to measure each of the control samples. Within site accuracies were found to be better than ± 10%, while comparison of the averages across sites were found to be within 3%. This demonstrates the ease of use of the MSIA Streptavidin D.A.R.T.'S and their ability to provide accurate results for most users.

Conclusion

MSIA Streptavidin D.A.R.T.'S, when loaded with biotinylated antibody, are able to specifically, efficiently, and reproducibly extract and enrich targeted proteins (in this case IGF1) from samples. The derivatization of proprietary monolithic columns to provide MSIA Streptavidin D.A.R.T.'S gives users the flexibility to tailor the tips with their own specific biotin-conjugated affinity reagents. The results shown provide evidence to the high degree of performance that is offered by MSIA Streptavidin D.A.R.T.'S.

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Ordering Information

MSIA D.A.R.T'S for Immunoaffinity Capture		
Compatible with the Thermo Scientific Versette Automated Liquid Handler and Thermo Scientific Finnpipette Novus i 12-Channel Electronic Pipette.		
Cat. No.	**Description**	**Packaging**
991STR11	MSIA Streptavidin D.A.R.T.'S	Pack of 96 tips
991STR12	MSIA Streptavidin D.A.R.T.'S	Pack of 24 tips
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		
Cat. No.	**Description**	
650-01-BS	Versette Automated Liquid Handler*	
Multichannel Pipettes and Pipette Stand		
Cat. No.	**Description**	**Quantity**
991SP12	Finnpipette Novus i Electronic 12-Channel Pipette and Adjustable Pipette Stand	1 pipette and 1 pipette stand

**Base unit only. Additional parts required.*

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