thermo scientific

Thermo Scientific MSIA Technology Application eBook

> Pharma/biopharma
> Clinical/translational research
> Sports anti-doping

ThermoFisher SCIENTIFIC

Collection of application notes

This collection of application notes is designed to provide insight into how Thermo Scientific™ MSIA™ technology can overcome common challenges associated with affinity purification. The next two pages will serve as an introduction into the technology, and then the book will explore why it's an efficient and reliable platform for large molecule characterization for a range of applications and industries.

Pharma/biopharma applications

Sports anti-doping applications

Clinical/translational research applications

Next generation large molecule bioanalysis simplified

Sample preparation and extraction steps for the quantification of large molecules can be complicated and laborious to optimize due to the complexity of biological samples which contain numerous background proteins and peptides. Furthermore, the analysis of such samples poses an additional challenge due to background peptides and proteins competing with the large molecule of interest, creating interference problems and ultimately impacting data accuracy.

Problem

As a result, biopharmaceutical, CROs, translational, clinical and sports anti-doping labs performing high throughput characterization of large molecules face several challenges:

> Insufficient sensitivity at low levels of detection (picogram quantities)

> High background noise and carryover

> Sub-standard data quality and reproducibility

> Low efficiency

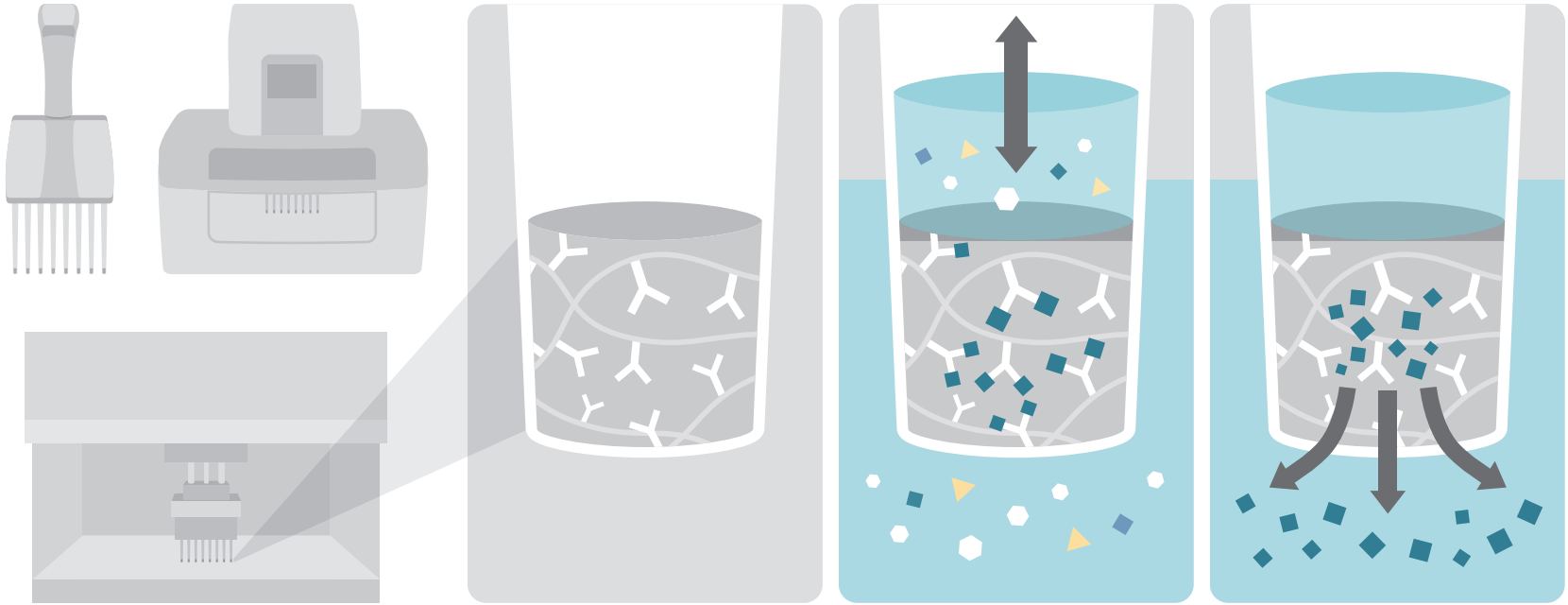
> Inability to standardize and transfer analytical methods between labs.

Solution

Thermo Scientific Mass Spectrometric Immunoassay (MSIA) technology represents a novel affinity capture method for large molecule bioanalysis. It enables users to affinity purify target analytes with ease from complex biological matrices for downstream analysis using different detection methods. MSIA technology is based on proprietary monolithic microcolumns that are densely coated with a target-specific affinity ligand for effective and efficient analytical affinity purification of even low-abundant target analytes. Housed within a pipette tip, these microcolumns create a versatile and user-friendly affinity capture device that provides the flexibility to analyze sample volumes from 10 µL to mL quantities and concentrations down to pg/mL levels.

MSIA technology streamlines large molecule bioanalysis with simple push button automation

The Thermo Scientific™ Versette™ automated liquid handler and the Tecan Freedom EVO® series with MCA96 liquid handling arm are available for analysis of target analytes in a high throughput 96-well format. Alternatively, the Thermo Scientific™ Finnpipette™ Novus i multichannel electronic pipette is ideal for low throughput or assay development work.



Automation of MSIA technology allows labs to meet productivity demands while reducing assay development timelines, supporting easy transfer for assays between labs, and eliminating user induced errors.

Quantitative analysis of an antibody drug conjugate using MSIA D.A.R.T.'S technology

> Although traditional LBAs can identify the presence of antibodies, they provide insufficient information for the measurement of DARs for ADCs. To demonstrate the ADC quantitative capability of the Thermo Scientific Streptavidin MSIA D.A.R.T.'S workflow, MSIA D.A.R.T.'S were used to capture and elute ADC before HRAM-MS detection using the Thermo Scientific™ Q Exactive™ Quadrupole-Orbitrap™ Mass Spectrometer, which has the advantage of being able to detect intact antibodies.

> Following data acquisition, users have different options for data analysis. In this study, two methods were demonstrated: deconvolution and the XIC method. Each method reduced the biological complexity of the ADC MS data.

> Total ADC and a single DAR species of the ADC were accurately quantified (Figure below; Table below), with coefficients of variation less than 15% and accuracy within 20%. Users can additionally benefit from automation of this workflow using the Thermo Scientific Versette automated liquid handling platform. Read more about the quantitative analysis of an ADC – download the full application note below.

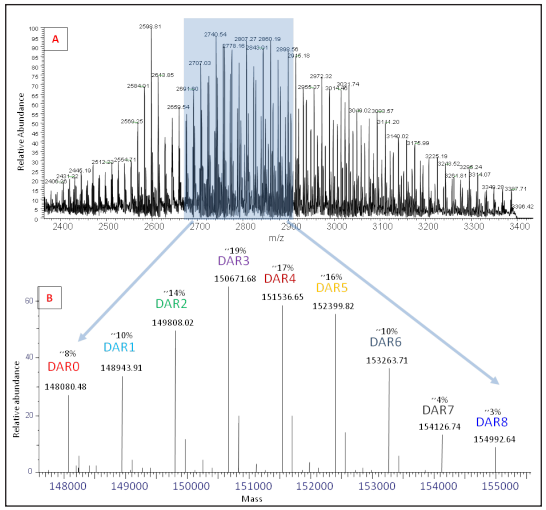


Figure: Results of LB-MSIA workflow performed on 20 µL sample containing 53.5 µg/mL ADC from rodent plasma.

DAR Species	% Total	Concentration (µp/ml)
0	7.81	7.81
1	9.62	9.62
2	14.20	14.20
3	18.58	18.58
4	16.81	16.81
5	16.04	16.04
6	10.44	10.44
7	3.95	3.95
8	2.55	2.55

Table: DAR species profile.

Learn more:

Webinar: Overcoming challenges in ADC bioanalysis
Smart note: Increased reproducibility and performance of hybrid immunoassay workflows compared to bead based approaches
Product specifications: MSIA Streptavidin D.A.R.T.'S product specification

Qualitative analysis of an antibody drug conjugate using MSIA D.A.R.T.'S technology

> Therapeutic antibodies and antibody-drug conjugates (ADCs) form a key part of next generation medicine development. The universal LB-MSIA workflow utilizing Streptavidin MSIA D.A.R.T.'S with biotin-conjugated anti-human IgG Fc provides an unmatched, highly sensitive, robust and reproducible method for the generation of high value data content for the bioanalysis of an ADC.

> The workflow successfully identified nine DAR (drug-to-antibody ratio) species within each ADC sample (Figure below).

> The sensitivity of the method was tested achieving a dosing curve dynamic range of 2.5-320 µg/mL of ADC with a linear regression of R^2 = 0.9978.

> The potential for the ADC to undergo biotransformation was identified by an in vitro stability study in which rodent plasma was incubated at 37 °C for 24 hours.

> This study demonstrates the flexibility and reliability you can benefit from by employing the MSIA Streptavidin D.A.R.T.'S workflow for your ADC analysis.

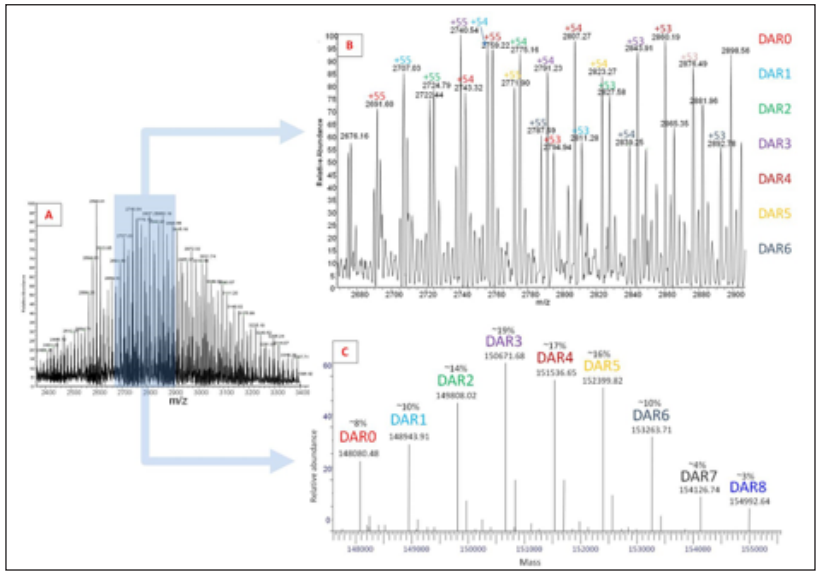


Figure: Results of the LB-MSIA workflow performed on a 20µL sample containing 53.5 µg/mL of the ADC from rodent plasma.

A-B) Ion Chromatogram showing multiple charge states of seven DAR species from the ADC sample. Each DAR species represents a different number of attached drug conjugates to the carrier antibody. C) Deconvolution of the MS data from the same ADC sample resulting in a reduction in data complexity. The deconvolved data was able to identify a total of 9 DAR species.

Learn more:

Webinar: Overcoming challenges in ADC bioanalysis
Smart note: Increased reproducibility and performance of hybrid immunoassay workflows compared to bead based approaches
Product specifications: MSIA Streptavidin D.A.R.T.'S product specification

Pre-clinical analysis of therapeutic antibodies of differing allotypes in rodent plasma using MSIA D.A.R.T.'S technology

> The research and development of novel antibody therapeutics requires structural information such as variants or different sites of glycosylation and other post translational modifications. This type of analysis is often essential for testing drug safety, stability and efficacy.

> To demonstrate the effectiveness and consistency of MSIA D.A.R.T.'S technology, MSIA Streptavidin D.A.R.T.'S were used to capture and elute humanized, fully human and chimeric therapeutic mAbs before analysis via LC-MS.

> The MSIA workflow demonstrated coefficients of variation and sensitivity akin to a conventional LBA, highlighting the reliability of this workflow for characterizing mAbs of different allotypes. Furthermore, the assay delivered the highly sensitive and reproducible detection of intact mAbs at concentrations as low as 31.25 ng/mL in rodent plasma, providing you with a robust solution for low-abundance mAb detection and characterization.

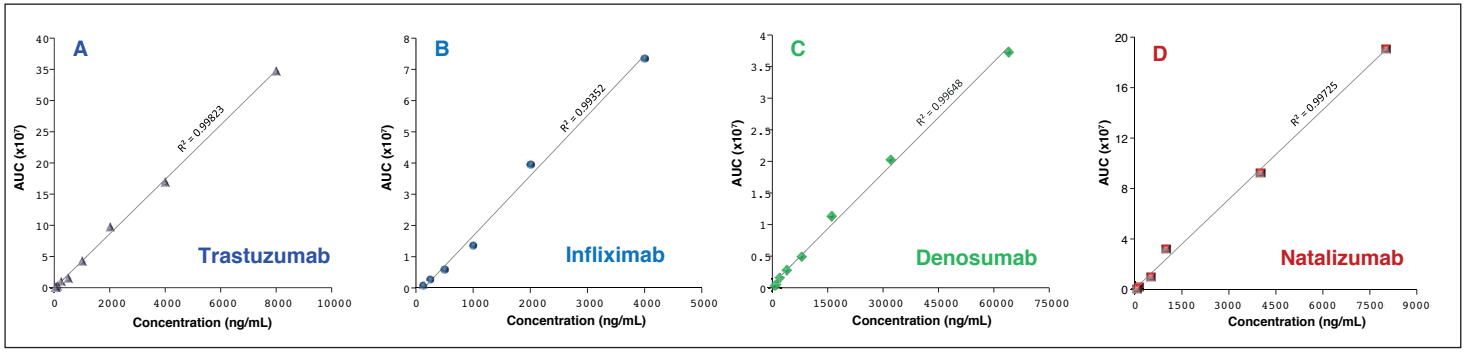


Figure: Results of the intact analysis of four therapeutic mAbs analyzed with the LB-MSIA workflow that spanned the following dynamic ranges: A) Dynamic range of trastuzumab: 31.25-8000 ng/mL B) Dynamic range of infliximab: 62.5-4000 ng/mL C) Dynamic range of denosumab: 500-64,000 ng/mL D) Dynamic range of natalizumab: 31.25-8000 ng/mL.

Learn more:

- **Webinar:** MSIA workflow for therapeutic antibody analysis
- **Application notes:** Ligand binding mass spectrometric immunoassay (LB-MSIA™) workflow with deglycosylation for therapeutic antibodies
- A universal pre-clinical solution for the bioanalysis of fully human therapeutic monoclonal antibodies in plasma
- MSIA workflow for therapeutic antibodies: qualitative, quantitative, and functional verification data from HR/AM detection of intact, reduced, and peptide-level forms of adalimumab
- **Smart note:** Increased reproducibility and performance of hybrid immunoassay workflows compared to bead based approaches
- **Product specifications:** MSIA Streptavidin D.A.R.T.'S product specification
- **Protocol:** MSIA Steptavidin D.A.R.T.'S
- **Technical Manual:** Demonstration of the LB-MSIA protocol for the analysis of intact adalimumab from mouse plasma

Pre-analytical deglycosylation of therapeutic antibodies further improves upon the ligand binding-MSIA workflow by decreasing data complexity and increasing sensitivity

> The MSIA workflow for therapeutic mAb bio-analysis is enabled by Streptavidin MSIA D.A.R.T.'S (Figure 1).

> Pre-analytical deglycosylation improves upon the ability to identify posttranslational modifications that may be present in complex in vivo biotransformation studies and drug:antibody ratio (DAR) determination.

> The use of the Q Exactive for HRAM detection helps provide additional analytical flexibility and data content over other developing triple quadrupole methods that are reliant on peptide analysis.

> The combined benefits of the hybrid approach enabled the characterization of a deglycosylated mAb over a wide dynamic range (20-5000 ng/mL), while maintaining coefficients of variation of <15% and accuracies within 20%. This represents an improvement over the 125 ng/mL limit-of-detection obtained with the non-deglycosylated intact workflow.

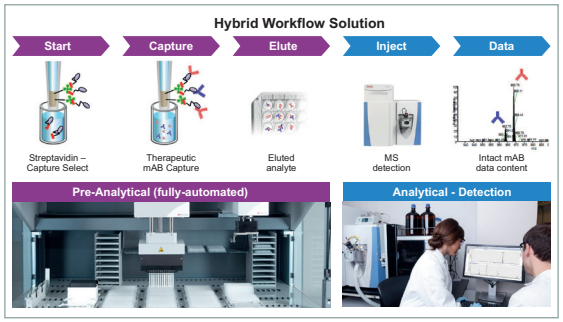


Figure 1: A schematic showing the five major steps of the LB-MSIA workflow.

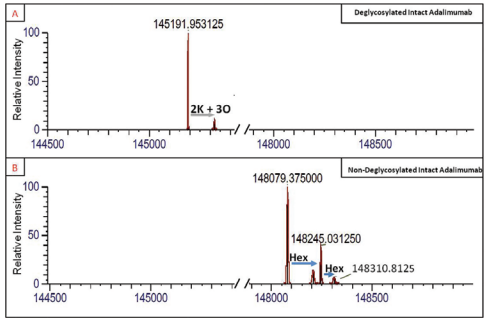


Figure 2 A). Deconvolved average mass (M+H) of deglycosylated intact adalimumab. B) Deconvolved average mass (M+H) of non-deglycosylated intact adalimumab.

Learn more:

- **Webinar:** MSIA workflow for therapeutic antibody analysis
- **Application notes:** Ligand binding mass spectrometric immunoassay (LB-MSIA™) workflow with deglycosylation for therapeutic antibodies
- A universal pre-clinical solution for the bioanalysis of fully human therapeutic monoclonal antibodies in plasma
- MSIA workflow for therapeutic antibodies: qualitative, quantitative, and functional verification data from HR/AM detection of intact, reduced, and peptide-level forms of adalimumab
- **Smart note:** Increased reproducibility and performance of hybrid immunoassay workflows compared to bead based approaches
- **Product specifications:** MSIA Streptavidin D.A.R.T.'S product specification
- **Protocol:** MSIA Steptavidin D.A.R.T.'S
- **Technical Manual:** Demonstration of the LB-MSIA protocol for the analysis of intact adalimumab from mouse plasma

Demonstrating the effectiveness of the MSIA Streptavidin workflow for the bioanalysis of fully human therapeutic mAbs using adalimumab in rodent plasma as a model biological system

> A unique hybrid solution for qualitative analysis of fully human intact therapeutic mAbs such as adalimumab in preclinical research, with the additional benefit of being amenable to automation.

> The Q Exactive hybrid quadrupole-Orbitrap mass spectrometer for HRAM detection offers users increased data content and analytical flexibility over other developing triple quadrupole platforms as it does not rely on peptide analysis.

> In this demonstration, intact native adalimumab mAb from rodent plasma was characterized over a wide dynamic range (125-8000 ng/mL). Coefficients of variation and sensitivity were akin to those in a traditional ligand binding assay (LBA), but MSIA technology brings the added benefits of providing a remedy for neutralization events and characterization information for the therapeutic mAbs in addition to high quality data and efficiency (Figure below).

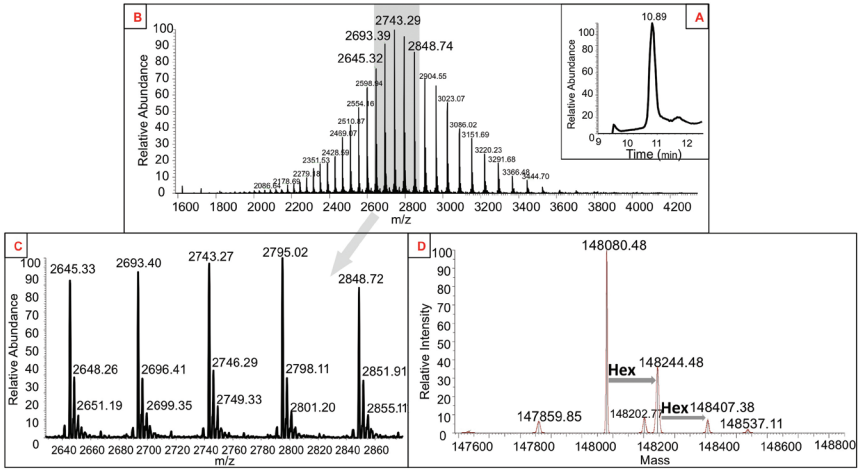


Figure: 500ng adalimumab sample at a concentration of 2.5 µg/mL purified from mouse plasma. A) Base peak chromatogram of adalimumab showing the elution profile of intact adalimumab with all of its sugars. B) and C) Raw MS spectra. D) Deconvolved average mass (M+H) of intact adalimumab.

Learn more:

- **Webinar:** MSIA workflow for therapeutic antibody analysis
- **Application notes:** Ligand binding mass spectrometric immunoassay (LB-MSIA™) workflow with deglycosylation for therapeutic antibodies
- A universal pre-clinical solution for the bioanalysis of fully human therapeutic monoclonal antibodies in plasma
- MSIA workflow for therapeutic antibodies: qualitative, quantitative, and functional verification data from HR/AM detection of intact, reduced, and peptide-level forms of adalimumab
- **Smart note:** Increased reproducibility and performance of hybrid immunoassay workflows compared to bead based approaches
- **Product specifications:** MSIA Streptavidin D.A.R.T.'S product specification
- **Protocol:** MSIA Steptavidin D.A.R.T.'S
- **Technical Manual:** Demonstration of the LB-MSIA protocol for the analysis of intact adalimumab from mouse plasma

Generating qualitative, quantitative and functional mAb data using the MSIA Streptavidin workflow with intact, reduced and peptide-level forms of adalimumab

> MSIA Workflow for Therapeutic Antibodies to measure three key properties – antibody quantity, quality and functionality – simultaneously.

> Streptavidin MSIA D.A.R.T.'S yielded a highly purified concentration of adalimumab, thereby reducing background and enhancing sensitivity.

> The bottom-up workflow provides analytical detection limit of 5 ng/mL for adalimumab from human donor plasma, using high flow LC. It also provides high peptide coverage: 91.1% peptide coverage of the adalimumab heavy chain sequence and 100% for the light chain.

> The reduced and alkylated workflow easily resolves intact heavy and light antibody chains in significantly shorter LC run times, with the light chain eluting at 7.49 minutes and the heavy chain at 8.66 minutes.

> The intact molecule workflow characterizes all molecular variants simultaneously: The Q Exactive MS system easily resolved the different isoforms of adalimumab, enabling researchers to quantify and detect, with essentially no sample preparation, drug-antibody ratios, post-translational modifications and other physical changes to the molecules in a single pass.

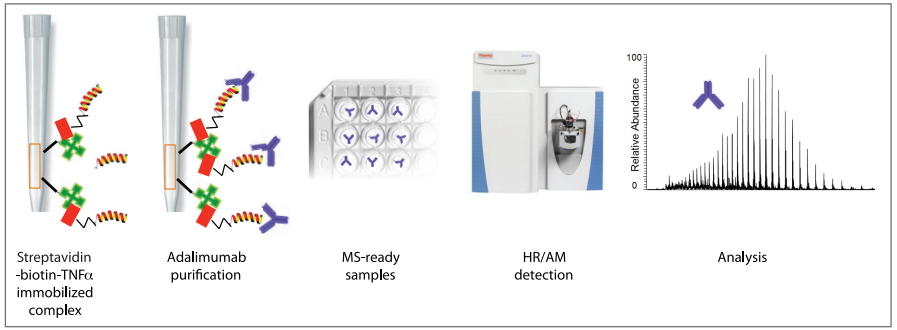


Figure 1. MSIA Workflow for Therapeutic Antibodies.

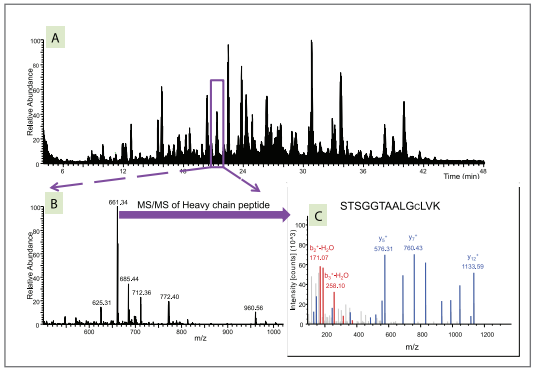


Figure 2. LC-MS analysis of trypsin digested adalimumab (5ng/mL) extracted from 200 µL of plasma. (A) Total ion chromatogram, (B) mass spectrum of peptide ions that were eluted around 21 minutes in the chromatogram shown in (A), and (C) MS/MS and amino acid sequence of heavy chain peptide at m/z 661.34.

Learn more:

- **Webinar:** MSIA workflow for therapeutic antibody analysis
- **Application notes:** Ligand binding mass spectrometric immunoassay (LB-MSIA™) workflow with deglycosylation for therapeutic antibodies
- A universal pre-clinical solution for the bioanalysis of fully human therapeutic monoclonal antibodies in plasma
- **Smart note:** Increased reproducibility and performance of hybrid immunoassay workflows compared to bead based approaches
- **Product specifications:** MSIA Streptavidin D.A.R.T.'S product specification
- **Protocol:** MSIA Steptavidin D.A.R.T.'S
- **Technical Manual:** Demonstration of the LB-MSIA protocol for the analysis of intact adalimumab from mouse plasma

Quantitative analysis of intact therapeutic mAbs of different allotypes using the MSIA Streptavidin EVO workflow

> The Thermo Scientific MSIA Streptavidin EVO workflow for Tecan's Freedom EVO instrument utilizes MSIA Streptavidin EVO microcolumns with biotin-conjugated anti-IgG Fc to efficiently and robustly quantify therapeutic mAbs of different human IgG subclasses.

> The affinity purified mAb eluates were separated and MS data were acquired using the Q Exactive Plus instrument. HRAM detection helped provide additional analytical flexibility and data content by providing, in addition to the quantitative data, characterization data of intact therapeutic mAbs.

> The following mAbs of different allotype subclasses were analyzed: adalimumab (Humira®), infliximab (Remicade), natalizumab (Tysabri) and trastuzumab (Herceptin) (Figure 2, next page).

> Coefficients of variation were less than 20% and accuracies were within 19%, allowing you to feel confident about your results. You can also benefit from the dynamic range of quantification offered by this workflow, spanning over three orders of magnitude. This technology is designed for researchers in biopharmaceuticals, clinical and translational research who are interested in quantitative mAb analysis.

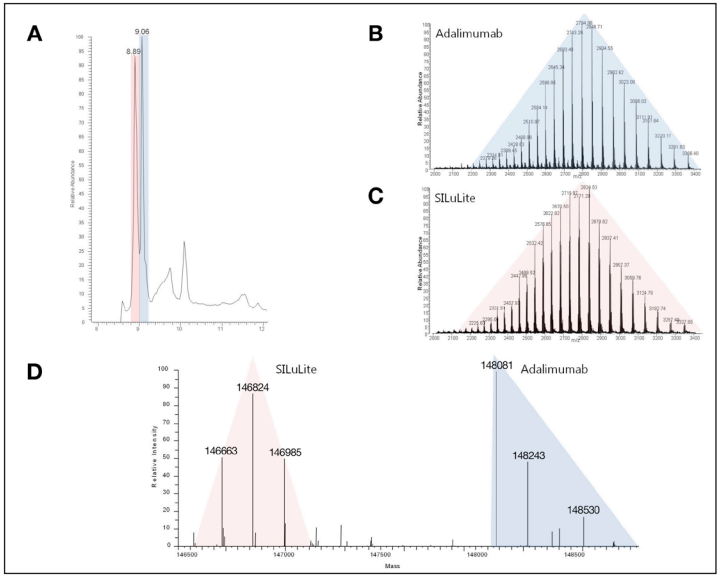


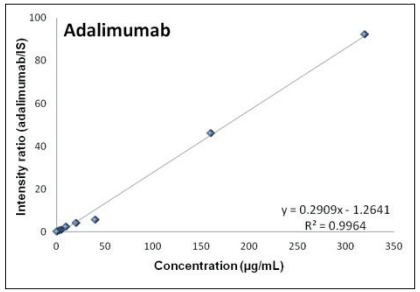
Figure 1: Results of the LB-MSIA workflow performed on a 20 µL rodent plasma sample containing 10 µg/mL of adalimumab and 35 µg/mL SILuLite. A) Total ion chromatogram of the adalimumab sample showing the elution profile of intact adalimumab and SILuLite IS. B) Mass spectrum showing multiple charge states of adalimumab from the sample. C) Mass spectrum showing multiple charge states of the SILuLite IS from the sample. D) Deconvolved spectrum showing the masses and peak intensities for SILuLite (IS) (146824 Da) and adalimumab (148081).

Continued on next page

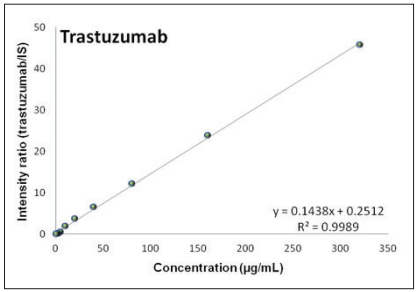
Learn more:

Application note: Qualitative analysis of therapeutic antibodies of differing allotypes in rodent plasma
Technical Manual: Protocol for the use of MSIA™ Streptavidin-EVO
Brochure: Thermo Scientific MSIA microcolumns for Freedom EVO platform

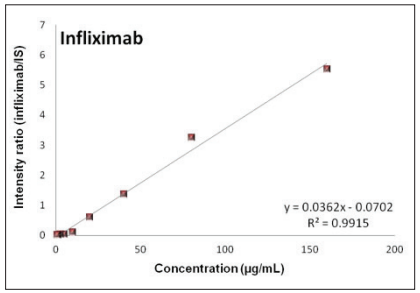
Quantitative analysis of intact therapeutic mAbs of different allotypes using the MSIA Streptavidin EVO – *continued*



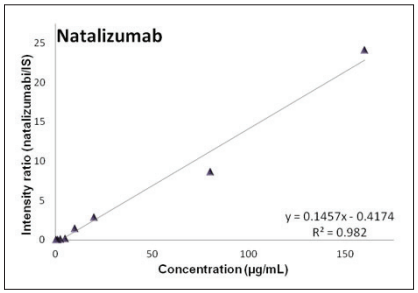
Adalimumab Control Sets			
Actual Conc. (µg/mL)	Experimental Conc. (µg/mL)	CV (%) n=5	Accuracy (%) n=5
120	97.7	13	-19
15	12.8	9	-15
3.75	4.1	8	8



Trastuzumab Control Sets			
Actual Conc (µg/mL)	Experimental Conc. (µg/mL)	CV (%) n=5	Accuracy (%) n=5
120	110.5	7	-8
15	15.7	17	5
3.75	3.3	11	-13



Infliximab Control Sets			
Actual Conc. (µg/mL)	Experimental Conc. (µg/mL)	CV (%) n=5	Accuracy (%) n=5
120	104.7	11	-13
15	13.6	5	-9
3.75	3.4	5	-10



Natalizumab Control Sets			
Actual Conc. (µg/mL)	Experimental Conc. (µg/mL)	CV (%) n=5	Accuracy (%) n=5
120	110.5	7	-8
15	15.7	17	5
3.75	3.3	11	-13

Figure 2: Deconvolved quantitation of four therapeutic mAbs analyzed with LB-MSIA from rodent plasma over a discontinuous period of three days.

Learn more:

- **Application note:** Qualitative analysis of therapeutic antibodies of differing allotypes in rodent plasma
- **Technical Manual:** Protocol for the use of MSIA™ Streptavidin-EVO
- **Brochure:** Thermo Scientific MSIA microcolumns for Freedom EVO platform

Demonstrating the effectiveness of the MSIA Streptavidin EVO workflow for sensitive, reliable and automated analysis of therapeutic mAbs of differing allotypes

> Designed to bring the novel Thermo Scientific MSIA technology to Tecan's Freedom EVO series of robotic platforms equipped with a 96 multichannel arm.

> MSIA Streptavidin EVO microcolumns designed for Tecan Freedom EVO platform along with high selectivity of the CaptureSelect biotin-conjugated anti-IgG Fc provide an unmatched, highly sensitive, robust, and reproducible method for the generation of high value data content for the bioanalysis of each of the fully human, humanized and chimeric therapeutic antibodies with differing allotypes.

> The workflow enabled the characterization of multiple intact mAbs over a wide dynamic range capable of three orders of magnitude.

> Sensitivity and coefficients of variation akin to conventional LBAs were achieved for adalimumab (Humira), infliximab (Remicade), denosumab (Prolia), natalizumab (Tysabri) and trastuzumab (Herceptin). Intact mAb at concentrations as low as 244 ng/mL was detected in mouse plasma (Figure below). These results illustrate how users can simultaneously benefit from full automation and highly sensitive and reliable analysis of mAbs using the MSIA Streptavidin-EVO workflow.

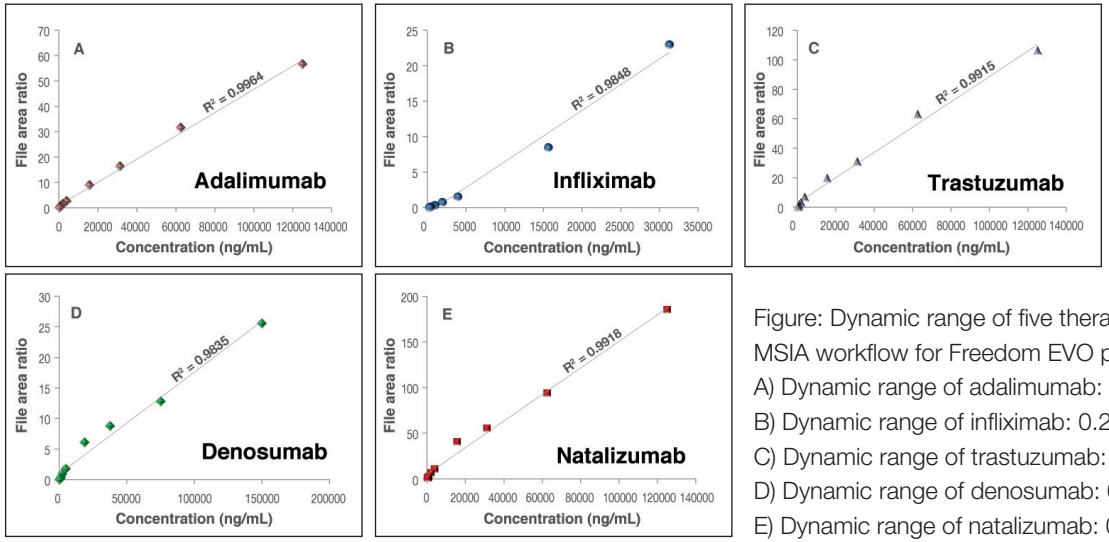


Figure: Dynamic range of five therapeutic mAb analyzed with LB-MSIA workflow for Freedom EVO platform:
A) Dynamic range of adalimumab: 0.244–125 µg/mL
B) Dynamic range of infliximab: 0.244–31.25 µg/mL
C) Dynamic range of trastuzumab: 0.244–125 µg/mL
D) Dynamic range of denosumab: 0.244–25 µg/mL
E) Dynamic range of natalizumab: 0.244–125 µg/mL

Learn more:

Application note: Qualitative analysis of therapeutic antibodies of differing allotypes in rodent plasma
Technical Manual: Protocol for the use of MSIA™ Streptavidin-EVO
Brochure: Thermo Scientific MSIA microcolumns for Freedom EVO platform

Insulin MSIA D.A.R.T.'S for more efficient targeted multiplexed insulin analogues detection and quantification

> Thermo Scientific Insulin MSIA D.A.R.T.'S address the analytical requirements of routine high-throughput, automated detection and simultaneous quantification of insulin and its analogues (pg/mL levels) in complex biological matrices.

> By employing Insulin MSIA D.A.R.T.'S for insulin capture and elution, background noise was reduced and selectivity was significantly increased, allowing Thermo Scientific™ Pinpoint™ software to fully analyze MS data.

> Three co-eluting insulin analogs at low concentrations were efficiently separated using LC-MS methods, particularly HRAM, demonstrating the sensitivity of this workflow (Figure 1). Furthermore, high quality quantitation data was obtained at these low concentrations. Detection and quantification ranges: 1.5 to 960 pM in a plasma matrix.

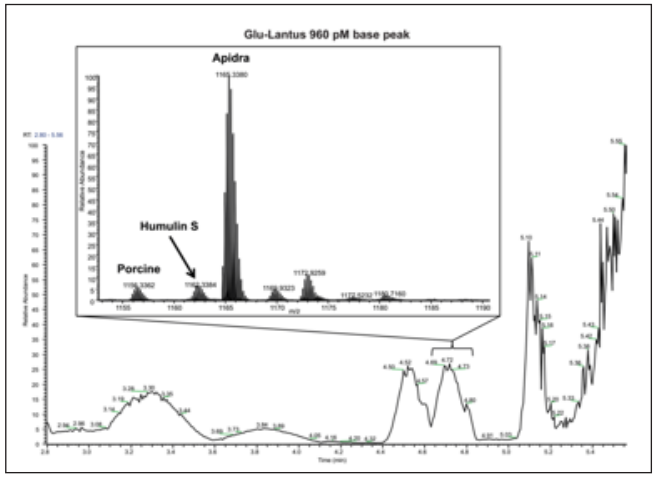


Figure 1: Base peak chromatogram for the MSIA-extracted human plasma sample spiked with 960 pM of both Apidra and Lantus insulin variants and 50 pM of porcine insulin (internal standard). The inset shows the summed mass range covering three of the four insulin variants.

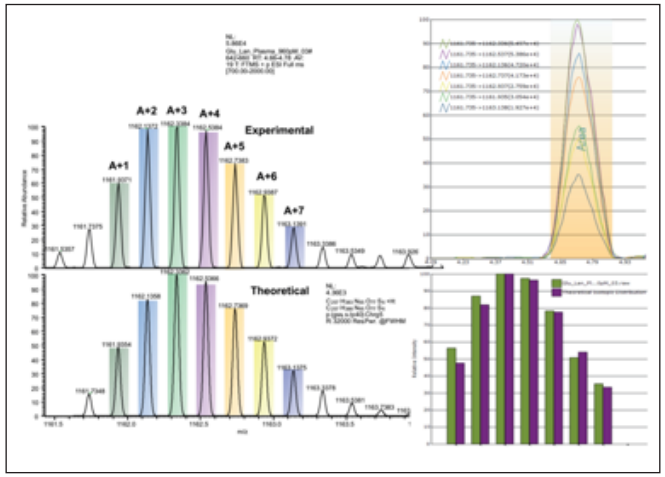


Figure 2. Data processing using Pinpoint software. Figure 2a shows the targeted data extraction based on isotopic m/z values for the seven most abundant isotopes, and a ±7 ppm extraction tolerance based on the theoretical isotopic distribution. Figure 2b shows the overlaid extracted ion currents (XICs) for each of the targeted isotopes. The AUC values for each isotope were used to evaluate the scoring shown in Figure 2c, where the relative AUC values for the collective isotopic distribution were compared to the theoretical value.

Learn more:

Technical Manual: MSIA Insulin D.A.R.T.'S

Protocol: Demonstration protocol for affinity purification and analysis of human insulin from human plasma

Robust immunoenrichment and reproducibility across multiple labs

> The reproducibility of Streptavidin MSIA D.A.R.T.'s to enable the specific extraction and enrichment of a targeted protein from biological samples was tested in an Insulin-Like Grown Factor (IGF1) model.

> Performance was evaluated across five beta-test sites through the use of a biotin-conjugated anti-human IGF1 antibody and the developed IGF1 MSIA-SRM. Results showed that accuracy was better than ± 10%, and within 3% across sites (Table below). These further iterate the ability of MSIA technology to provide simple, consistent and accurate results.

> This model system serves as a template for future LC/LC-MS methods that perform quantitative immunoaffinity proteomics from biological samples.

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: With Streptavidin MSIA D.A.R.T.'s, multiple users can obtain similar results, as demonstrated by the 2-3% CVs and the high accuracies for measuring the control samples across multiple sites.

Learn more:

- **Application notes:** Comparative performance evaluation of the mass spectrometric immunoassay (MSIA™) and magnetic bead formats (IGF1 using protein A/G MSIA D.A.R.T.'S)
- Demonstration of performance characteristics of protein A, G and A/G MSIA™ D.A.R.T.'S (IGF1)
- A universal mass spectrometric immunoassay (MSIA™) model system based on human insulin-like growth factor 1
- **Brochure:** Mass spectrometric immunoassay (MSIA™) pipette tips
- **Protocol:** Demonstration protocol for affinity purification and LC/MS analysis of digested IGF-1 from human plasma

Practical, broadly applicable workflow for rapid development of MS-based SRM methods for translational research

> Demonstration of practical, scalable method for rapid development of MS-based SRM methods for sixteen proteins [Apolipoprotein family (ApoE, ApoA1, ApoCI, ApoCIII, and ApoJ), medium- to high-abundance proteins (ceruloplasmin, vitamin D binding protein, beta-2 microglobulin and C-reactive protein), and low-abundance proteins (procalcitonin, parathyroid hormone, insulin-like growth factor 1, prostate-specific antigen, erythropoietin, proprotein convertase subtilisin/kexin type 9, and amyloid beta)], which are of importance to clinical research.

> Extracted ion chromatograms of MSIA-SRM analysis of biological (donor) samples were free of significant interferences.

> Detect and quantify target proteins at pg/mL to mg/mL within established useful ranges.

> Serial MSIA extraction demonstrated similar recovery to performing each MSIA extraction individually, allowing users to conserve sample while multiplexing, and eliminating the need to determine optimal antibody ratios per sample for faster process development. (Figure below).

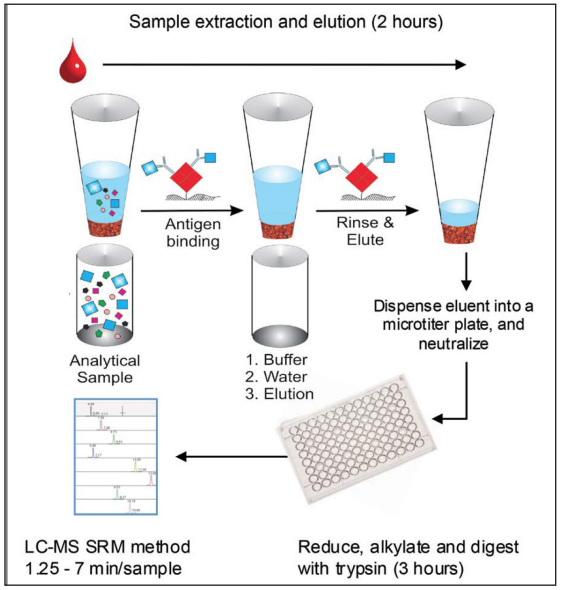


Figure: High throughput MSIA-SRM workflow

Comparing the performance of the MSIA and magnetic bead format

> Thermo Scientific MSIA D.A.R.T.'S are designed to offer enhanced sensitivity and accuracy, as well as efficiency. A head-to-head comparison of Protein A/G MSIA™ D.A.R.T.'S against traditional Protein A/G magnetic beads specifically examining the lower limits of detection and quantification (LLOD and LLOQ, respectively), as well as non-specific binding of both analyte extraction formats was performed using an IGF1 model system.

> Protein A/G MSIA D.A.R.T.'S exhibited superior lower limits of detection and quantification (LLOD & LLOQ, respectively) over BioClone™ Protein A/G BcMag™ magnetic beads. Specifically, LLOD & LLOQ were 10- and 20-fold increased, respectively, and this was attributed to higher quality immuno-affinity purification that resulted in less non-specific binding and therefore less contaminant carry-over (Figures 1-2). This resulted in a 55% increase in IGF1 selectivity over the beads.

> These technical benefits offer you high levels of confidence in the resulting data generated. In this study MSIA D.A.R.T.'S were used in combination with the Thermo Scientific Versette automated liquid handling platform. For small sample numbers or assay development, the Finnipipette Novus i Multichannel electronic pipette can also be used with MSIA D.A.R.T.'S.

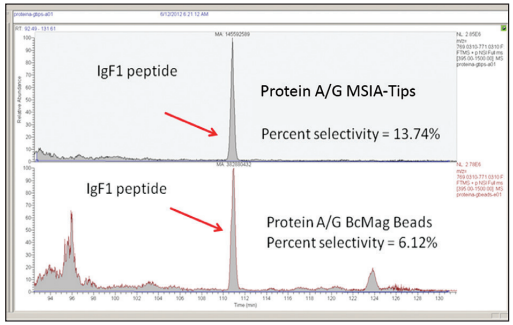


Figure 1. Resultant LTQ Orbitrap XL MS/MS data of IGF1 obtained from both the MSIA-Tips (Top panel) and BcMag Magnetic Bead (Bottom panel) protein capture and enrichment. Data clearly shows less interferences for the Tip extraction vs. the Bead when detecting the peptide of interest.

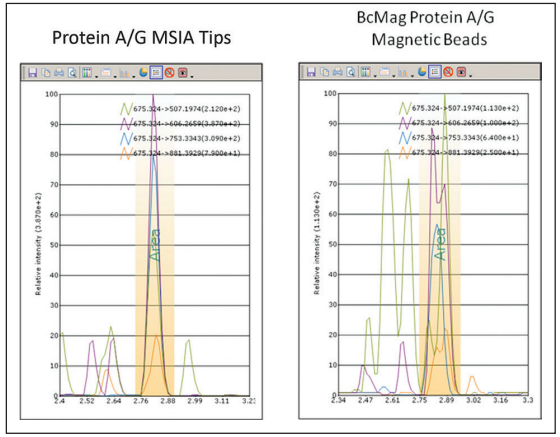


Figure 2. Representative data generated using the TSQ Vantage of the same IGF1 peptide. The tip extraction method resulted in more uniform peak shape and less interference within the elution window than with the beads.

Learn more:

- **Application note:** A universal mass spectrometric immunoassay (MSIA™) model system based on human insulin-like growth factor 1 (IGF1)
- Demonstration of performance characteristics of Protein A, G and A/G MSIA™-tips
- Thermo Scientific MSIA Streptavidin D.A.R.T.'S: robust immunoenrichment process and reproducibility across multiple labs
- **Brochure:** Thermo Scientific mass spectrometric immunoassay (MSIA) pipette tips
- **Protocol:** Demonstration protocol for affinity purification and LC/MS analysis of digested IGF-I from human plasma

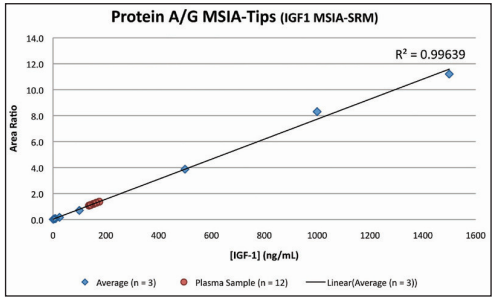
Demonstration of performance characteristics of Protein A, G and A/G MSIA D.A.R.T.'S

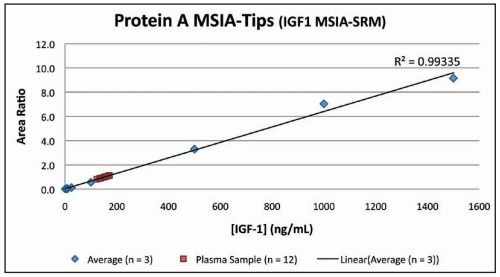
> The performance characteristics of MSIA D.A.R.T.'S (Protein A, Protein G, and Protein A/G) were tested using a model system MSIA-SRM, based on IGF1.

> All three MSIA D.A.R.T.'S displayed large dynamic ranges (1-1500 ng/mL; Figure below), protein recovery rates of >85 % and coefficients of variation ≤10 %.

> Limits-of-detection as low as 5.2 femtomoles of IGF1 from diluted samples were achieved, without any additional sample preparation or protein depletion (Table below).

> These MSIA D.A.R.T.'S format provides you with the flexibility to use your own specific antibodies for your applications. Furthermore, the functional pipette tip format allows you to address large or small sample cohorts using a Thermo Scientific Versette automated liquid handler or a Finnipipette Novus i Multichannel electronic pipette, respectively, thus cutting down labor time.





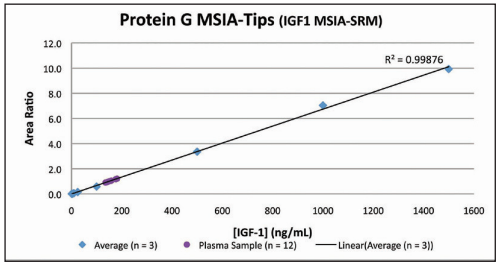


Figure: 8-point IGF1 calibration curves were generated from extraction and enrichment of IGF1 from a dilution series of IGF1 standard using Protein A/G MSIA-Tips.

MSIA-Tip Type	Protein A	Protein G	Protein A/G
LLOOD	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)*	10 - 1500 ng/mL (5.2 - 7800 femtomole)*	10 - 1500 ng/mL (5.2 - 7800 femtomole)*

*Amounts based on a 40 µL plasma sample volume.

Table: 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.

Learn more:

- **Application note:** Comparative performance evaluation of the mass spectrometric immunoassay (MSIA™) and magnetic bead formats
- A universal mass spectrometric immunoassay (MSIA™) model system based on human insulin-like growth factor 1 (IGF1)
- Thermo Scientific MSIA Streptavidin D.A.R.T.'S: robust immunoenrichment process and reproducibility across multiple labs
- **Brochure:** Thermo Scientific mass spectrometric immunoassay (MSIA) pipette tips
- **Protocol:** Demonstration protocol for affinity purification and LC/MS analysis of digested IGF-I from human plasma

Product information

MSIA microcolumns for immunoaffinity capture		
Compatible with Tecan Freedom EVO platform with MCA96 head		
Part #	**Description**	**Packaging**
992STR96	MSIA Streptavidin-EVO	Racked, pack of 96

MSIA D.A.R.T.'S for immunoaffinity capture		
Compatible with Thermo Scientific Versette Automated Liquid Handler & Thermo Scientific Finnipipette Novus i Multichannel Electronic Pipette		
Part #	**Description**	**Packaging**
991001096	MSIA D.A.R.T.'S, Insulin	Racked, pack of 96
991001024	MSIA D.A.R.T.'S, Insulin	Blister package, pack of 24
991CUS02	MSIA D.A.R.T.'S, Custom	Racked, pack of 96
991R	Reloadable rack	1 reloadable rack, MSIA D.A.R.T.'S not included
991PRT11	MSIA D.A.R.T.'S, Protein A	Racked, pack of 96
991PRT12	MSIA D.A.R.T.'S, Protein A	Blister package, pack of 24
991PRT13	MSIA D.A.R.T.'S, Protein G	Racked, pack of 96
991PRT14	MSIA D.A.R.T.'S, Protein G	Blister package, pack of 24
991PRT15	MSIA D.A.R.T.'S, Protein A/G	Racked, pack of 96
991PRT16	MSIA D.A.R.T.'S, Protein A/G	Blister package, pack of 24
991STR11	MSIA D.A.R.T.'S, Streptavidin	Racked, pack of 96
991STR12	MSIA D.A.R.T.'S, Streptavidin	Blister package, pack of 24

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

Find out more at **thermofisher.com/msia**

ThermoFisher SCIENTIFIC