Technical Manual

Thermo Scientific
MSIA Streptavidin D.A.R.T.'S

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Thermo Scientific MSIA Streptavidin D.A.R.T.'S and Workflows: Forward MSIA-Streptavidin (Streptavidin→Biotin-Antibody→Protein) Reverse MSIA-Streptavidin (Streptavidin→Biotin-Antigen→Antibody therapeutics)

Product Information:

Thermo Scientific™ MSIA™ Streptavidin D.A.R.T.'S®		
Compatible with the Thermo Scientific™ Versette™ Automated Liquid Handler and Thermo Scientific™ Finnpipette® Novus™ i Electronic 12-Channel Pipettes (for immuno-precipitation).		
Cat. No.	*Description*	*Packaging*
991STR11	300 µl MSIA Streptavidin D.A.R.T.'S	Pack of 96 tips
991STR12	300 µl MSIA Streptavidin D.A.R.T.'S	Pack of 24 tips

Storage

Upon receipt, store at 4 °C. Product shipped with an ice pack.

Disclaimer

These products are supplied for life science research use only. They are not intended for medicinal, diagnostic or therapeutic use.

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Introduction

The Thermo Scientific Streptavidin-based Mass Spectrometric Immunoassay (MSIA) approach provides a fast, convenient and highly reproducible method for both manual and automated liquid handler enrichment of target analytes for subsequent mass spectrometric detection. The Streptavidin MSIA workflow is versatile, enabling rapid analysis of target analytes and/or their therapeutic binding partners. Furthermore, MSIA Streptavidin D.A.R.T.'S are designed for use with automated instruments, thus enabling large-scale screening of multiple samples.

MSIA Streptavidin D.A.R.T.'S utilize the Thermo-Pierce recombinant form of streptavidin, originally isolated from *Streptomyces avidinii*. Affinity-purified recombinant streptavidin is immobilized onto MSIA D.A.R.T.'S through directed coupling, and can be used to bind biotinylated ligands. Streptavidin is tetrameric, having 4 potential biotin binding sites, and is highly resistant to denaturation by acids or proteolytic enzymes. Streptavidin has the advantage over avidin that it is carbohydrate-free and more resistant to denaturation by guanidine•HCl. Further, streptavidin generally has less non-specific binding than avidin due to this absence of carbohydrates and the difference in charge. These qualities make the streptavidin-biotin system ideal for analyte purification and amenable to downstream analysis by label-free mass spectrometry.

MSIA Streptavidin D.A.R.T.'S are convenient, ready-to-use devices that offer the end user the flexibility to customize the affinity microcolumn for specific target analyte applications by using biotinylated ligands (antibodies and proteins) in a forward or reverse approach to analyze one or more target antigens or antibodies, respectively. Forward MSIA Streptavidin (**Fig. 1**) enables traditional biotinylated-antibody stacking onto the streptavidin tetramer surface, shown in Step 1 of the process. A repetitive pipetting mixing cycle of the antibody-loaded MSIA Streptavidin D.A.R.T.'S with the prepared biological sample captures and enriches for desired target antigens (both wild type and internal reference) in Step

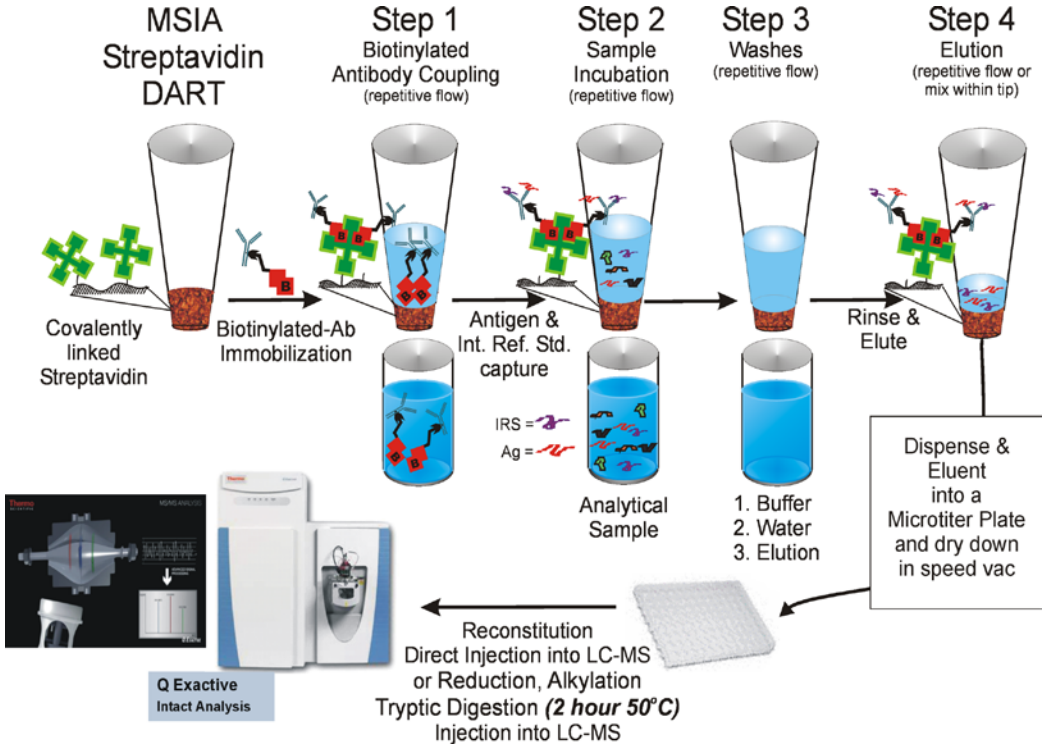


Fig. 1 Illustration of Forward MSIA Streptavidin approach. Step1) Biotin-conjugated antibody is coupled to MSIA Streptavidin D.A.R.T.'S. Step 2) MSIA Streptavidin D.A.R.T.'S. loaded with biotinylated antibody are used to capture and enrich target antigen (analyte) from biological sample. After tip washing of non-specifically bound impurities, the analyte is eluted and then detected by LC-MS analysis.

2. This allows for simultaneous purification and enrichment of the targeted analytes from typical biological matrices including serum, plasma, urine, cerebral spinal fluid, or cell culture supernatants. After sample incubation, the MSIA Streptavidin D.A.R.T.'S are rinsed (in Step 3) and the bound antigens are then dissociated from the MSIA Streptavidin D.A.R.T.'S using an elution buffer in Step 4. The elution buffer selection and the methodologies for mass spectrometric detection are both application- and target-specific. The eluted analytes are then directly injected into the LC-MS or further concentrated by a speed vacuum, subsequently reconstituted, and injected for intact analysis in a top-down approach. Alternatively, eluted analytes can be reduced, alkylated, and enzymatically digested in a bottom-up approach, which facilitates the analysis of high mass proteins.

Reverse Streptavidin MSIA, shown in **Fig. 2**, uses a biotin-conjugated antigen to capture and enrich for endogenous or therapeutic antibodies for their rapid detection and analysis by LC-MS. In this protocol biotinylated-protein or peptide antigens serve as the ligand coupled to the streptavidin base surface (Step 1). Affinity capture and enrichment of desired antibody (both therapeutic and internal reference) from the biological sample is accomplished in Step 2 using its antigen pair, loaded onto the MSIA Streptavidin D.A.R.T.'S. Affinity enrichment is typically applied to serum, plasma, urine, cerebral spinal fluid or cell culture supernatants. After sample incubation, the MSIA Streptavidin D.A.R.T.'S are rinsed in Step 3 to remove non-captured antibody and proteins. Bound antibody is eluted from the MSIA Streptavidin D.A.R.T.'S in Step 4 using an elution reagent. The elution reagent selection and the methodologies for mass spectrometric detection are target- and application-specific. The eluted antibody can be directly injected into the LC-MS for intact protein characterization or reduced, alkylated, and digested for bottom-up protein characterization. The Reverse MSIA Streptavidin approach can also be applied to enrich for other ligands that bind specifically to a biotinylated analytes.

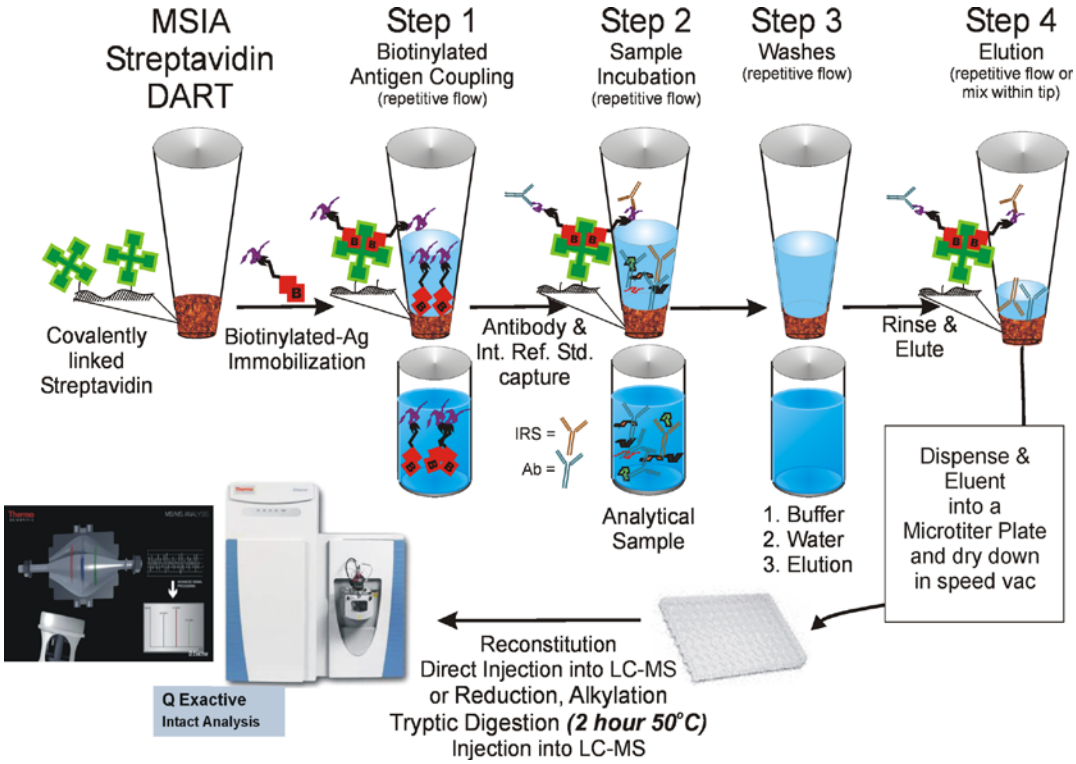


Fig. 2 Illustration of Reverse MSIA Streptavidin approach. MSIA Streptavidin D.A.R.T.'S are used to couple biotinylated antigen that bind target antibody (analyte) from biological sample. After tip washing of non-specifically bound impurities, the antibody is eluted and detected with LC-MS analysis.

Important Product Information

- Store the MSIA Streptavidin D.A.R.T.'S at 4 °C, do not freeze.
- This product is intended for single use only.
- MSIA Streptavidin D.A.R.T.'S are not intended for the transference or measurement of liquids. This product is intended for micro-scale analyte purification prior to mass spectrometric detection.
- For more information visit www.thermoscientific.com/msia.

Forward MSIA Streptavidin Protocol: Biotinylated Antibody Loading for Antigen extraction with Finnpipette Novus i Electronic 12-Channel Pipette

Note: The following information provided in this procedure describes the use of the MSIA Streptavidin D.A.R.T.'S in the semi-automated Finnpipette Novus i Electronic 12-Channel Pipette bench top/mobile platform format. The steps described can easily be translated to a fully automated Versette robotic platform (please contact local MSIA support for more details). These procedures are intended for **general guidance only** and may require further optimization by the end-user.

A. Additional Materials Required

- Finnpipette Novus i Electronic 12-Channel Pipette and Adjustable Pipette Stand (See Ordering Information)
- Assortment of Thermo Scientific™ Finnpipette®*F1 Adjustable-volume Pipettes
- Assortment of Thermo Scientific™ Finntip*Flex* Pipette Tips
- 96-well polypropylene microplates, 0.5mL, 1mL, or 2mL (Thermo Scientific Product No. 267334, 260251, 278743). Please note, we highly recommend using low-bind plastic ware to ensure efficient performance of the assay and prevent loss of analyte due to adsorption to the plastics.
- Fisher Chemical Optima LC/MS Water (Fisher Scientific Product No. W6-4).
- Dilution/Wash Buffer: Phosphate-buffered saline, pH 7.2 (PBS, Thermo Scientific Product No. 28372)

B. Alternate Equipment

- Versette Automated Liquid Handler (See Ordering Information)

C. Protocol Considerations

Biotinylated Antibody Loading

- Biotinylated antibodies used for enrichment with this product should have sufficient affinity for the analyte to ensure efficient capture of the analyte of interest. Antibodies for Enzyme Immunoassays (EIA) or Enzyme-Linked Immunosorbent Assay (ELISA) with suggested working dilutions of >1:8,000 are recommended. Generally, lower analyte concentrations necessitate greater antibody affinity constants (larger dilution factors for EIA or ELISA applications).
- Diluent/Wash Buffer can be prepared in bulk and stored at 4 °C until use.
- Buffers must be warmed to room temperature (25 ° C) prior to use for efficient binding.
- The prepared biotinylated antibody solution used in the loading phase should be thoroughly mixed in the Dilution Buffer by repeat inversion or using a rotating platform. **Aggressive agitation of biotinylated antibody solution will denature the antibody and result in loss of antibody function**.
- At a minimum, a single well of biotinylated antibody solution is necessary to perform each Streptavidin MSIA D.A.R.T.'S loading and analysis.
- Dedicate a row or column of the microtiter plate for assay development, thus allowing up to 8 (per column) or 12 (per row) samples to be addressed in parallel with the Finnpipette Novus i Electronic 12-Channel Pipette.

Sample Analyte Loading

- *Sample volume and concentration may be modified according to the end-user preference*. The sample dilution and volume are dictated by the biological matrix selected and the concentration of the analyte being targeted. If the analytical sample amount required exceeds the volume of the microtiter plate or micro-centrifuge tubes described in this protocol, then appropriately sized receptacles, such as deep well microtiter plates, may be substituted.
- *Cycle volume and number of cycle iterations may be modified according to the end-user preference*. Studies should be performed by end-user to ensure optimal extraction/purification of the end-user's targeted analyte by the biotinylated-antibody loaded MSIA Streptavidin D.A.R.T.'S.
- Thaw and/or warm samples to at least room temperature before preparation. A 37 °C water bath may be employed to expedite this process and ensure consistent sample temperature.
  Quick Tip: Centrifuge raw samples prior to aliquot distribution to ensure the removal of any particulates or debris that may be present, which may clog the distal tip microcolumn.
    - Additional substances such as detergents, chaotropic agents or protein carries, e.g., Tween-20 Detergent (Thermo Scientific Product No. TA-125-TW) or Bovine Serum Albumin (10g/L concentration) can be added to each analytical sample to help prevent the antigen from being absorbed or "captured" by the plastic walls of the microtiter plate used to house the sample. These are suggested additions and the use of detergents can also reduce the ability of the antibody to bind its antigen, but ultimately specific concentrations and substances can be modified by the end user per their specific requirements. **Note: Do not use Tween-20 in Thermo Scientific™ Q-Exactive™ or other trap-based instrument applications as this polymeric detergent will potentially contaminant the instrument.**
- Use the Dilution Buffer to serially dilute the analytical sample. Thoroughly mix prior to the next dilution.
- An appropriate internal reference standard (IRS) may be added at a known concentration to the biological matrix (analytical sample) prior to antigen loading onto the MSIA Streptavidin D.A.R.T.'S. Generally, select an IRS with the same epitope as the endogenous antigen and that has a mass shift from the endogenous form. Ultimately, the IRS is application specific, and the user should empirically determine the appropriate concentration and standard. When the IRS is added during the CAPTURE step, it can be used to normalize losses throughout the sample-prep process.
- An elution solvent must sufficiently disrupt the antibody/antigen complex. Some examples of elution solvents include acid (0. 2-1% trifluoroacetic acid or formic acid) in 0-40% acetonitrile or isopropanol, base buffer (ammonium carbonate or Tris buffer pH 9-11) in 0-40% organic (acetonitrile, isopropanol), and denaturants (2-6M guanidine hydrochloride, 6-8M Urea). Care should be taken to not use an elution solvent that causes the captured analyte to precipitate within the MSIA Streptavidin D.A.R.T.'S.
  Quick Tip: To reduce loss of the eluted analyte to absorption to plastic or glassware, a blocking agent (e. g., 15 mg/L glucagon, adrenocorticotropic hormone (ACTH) or bovine serum albumin (BSA) in the elution solvent) may be used. Care should be taken in selecting a blocking agent that does not negatively affect downstream processes or detection of the targeted analyte.
- Transfer the eluent directly from the MSIA Streptavidin D.A.R.T.'S to the container that will be used in further processing or analysis to avoid loss of the eluent.

D. Forward MSIA Streptavidin Protocol

1. Dilute the biotinylated antibody with the Dilution Buffer for a final concentration range of 0.02 – 0.1 mg/mL. Add 125 µL of this solution into each well of the microtiter plate. At a minimum a single well of biotinylated antibody solution is necessary to perform each MSIA Streptavidin D.A.R.T.'S loading and analysis. Generally a row or column of the microtiter plate is assessed for assay development, thus allowing up to 8 (per column) or 12 (per row) samples to be addressed in parallel with the Finnpipette Novus i Electronic 12-Channel Pipette.
2. Load a well of Wash Buffer (200 µL) for each MSIA Streptavidin MSIA D.A.R.T.'S used.
   Quick Tip: Dedicate a row or column of Wash Buffer in the microtiter plate for assay development.
   Note: Larger volumes can be used with 2mL deep-well plates and by adjusting the pipette tip and stand heights.
3. If Finnpipette Novus i Electronic 12-Channel Pipette and Adjustable Pipette Stand (sold separately) is used, snap on multichannel pipette bracket to Finnpipette Novus i Electronic 12-Channel Pipette.

4. Securely affix MSIA Streptavidin D.A.R.T.'S onto the Finnpipette Novus i Electronic 12-Channel Pipette nose cone. Mount the Finnpipette Novus i Electronic 12-Channel Pipette onto its stand, raise pipette up from the deck. Insert microtiter plate onto the stand deck.
5. The general workflow for biotinylated antibody loading is presented in **Table 1**. The pipetting cycle iterations are performed by methodically aspirating and dispensing (standard pipette mixing action) using the listed cycle volumes provided. *The numbers of iterations and Finnpipette Novus i Electronic 12-Channel Pipette speed setting provided are a general guideline and may require further optimization (increased or decreased) by the end-user.*

Table 1. Biotinylated Antibody Loading/Workflow for Finnpipette Novus i Electronic 12-Channel Pipette using a speed setting of 8 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Biotinylated Ab Solution	125	100	~400	14 minutes
3	Wash Buffer	200	175	10	20 seconds

6. Turn on Finnpipette Novus i Electronic 12-Channel Pipette by pressing the trigger button once.
7. Press Menu (left selection key) and scroll the function list with scroll key and select MSIA function with OK (right selection key).
8. Position the sample plate under the pipette. Lower the MSIA Streptavidin D.A.R.T.'S into the Wash Buffer reservoirs using the slide vertical control knob on the stand. Ensure that the open end remains completely immersed in the volume of liquid during each step of the workflow to maintain proper sample and wash flow through the MSIA Streptavidin D.A.R.T.'S. Failure to do so will result in aspiration of air and inconsistency in tip treatment. Also, do not press the MSIA Streptavidin D.A.R.T.'S into the bottom of the liquid holding vesicle as this will restrict flow.
9. Pre-rinse MSIA Streptavidin D.A.R.T.'S with Wash Buffer prior to loading with biotinylated antibody. Select the WASH function on the Finnpipette Novus i Electronic 12-Channel Pipette. The WASH submenu appears with preferred default values for the three variables of SPEED, VOLUME and number of CYCLES. Please adjust the speed, volume and mixing cycles to match those described in **Table 1** by pressing the edit button.
   Quick Tip: Nine PROGRAM submenus under the MSIA menu can be setup with user defined values and saved with preferred SPEED, VOLUME and CYCLE numbers using the pipette scroll key and selection keys.
   Quick Tip: Any program may be interrupted by selecting CANCEL (left selection key), which will stop the run.
10. Press the trigger button **once**, located on the back of the pipette handle, to begin rinsing of the tips with Wash Buffer. Immediately, the Finnpipette Novus i Electronic 12-Channel Pipette will begin mixing and the cycle numbers will start counting down. When finished the Finnpipette Novus i Electronic 12-Channel Pipette will indicate completion of the WASH step by requiring the user to depress the trigger to perform the "BLOWOUT."
11. To BLOWOUT, raise the Finnpipette Novus i Electronic 12-Channel Pipette so that the distal ends of the MSIA Streptavidin D.A.R.T.'S above the liquid level, yet still remaining within the wells of the microtiter plate. Depress the trigger once to allow the Finnpipette Novus i Electronic 12-Channel Pipette to dispense any remaining liquid from the tips.
12. Load MSIA Streptavidin D.A.R.T.'S with biotinylated antibody. Scroll the function list and select CAPTURE with OK (right selection key). The CAPTURE submenu appears with preferred default values for the three variables of SPEED, VOLUME and number of CYCLES. Please adjust the speed, volume and mixing cycles to the values in **Table 1** by pressing the edit button.
13. Lower the MSIA Streptavidin D.A.R.T.'S into the biotinylated antibody solution, again ensuring the distal ends of the tips are not pressed against the bottom of the microtiter wells, restricting the flow during pipetting.
14. Press trigger button; cycling starts and the cycle number begins to count-down.

15. After completing CAPTURE step, lift MSIA Streptavidin D.A.R.T.'S above the samples and press trigger to the BLOWOUT function.
16. Rinse the biotinylated-antibody-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer. Select the WASH function on the Finnpipette Novus i Electronic 12-Channel Pipette, immerse the tips into the wash buffer pre-loaded into microtiter wells, and then press the trigger to begin rinsing of the tips with Wash Buffer. Please edit the values to match those in **Table 1**.
17. Raise the tips above the Wash Buffer and press trigger once again to perform the BLOWOUT function.
18. Once the MSIA Streptavidin D.A.R.T.'S are loaded with biotinylated antibody, they can be immediately used for your target analyte purification.
19. Dilute the analyte-containing biological matrix with a known volume of Dilution Buffer and internal reference standard (if applicable). Mix the diluted sample thoroughly via repeat inversion, vortexing or rotary platform. Dispense a measured aliquot of the analytical sample into an individual well of a microtiter plate or a micro-centrifuge tube.
20. Load a well of Wash Buffer (200 µL) and corresponding wells of water (200 µL per) required for each MSIA Streptavidin D.A.R.T.'S used. Washing removes non-specifically bound impurities.
    Quick Tip: Dedicate a row or column of Wash Buffer and Water in the microtiter plate for assay development.
    Note: Larger volumes can be used with 2mL deep-well plates and by adjusting the pipette tip and stand heights.
21. The mechanical application of the MSIA Streptavidin D.A.R.T.'S in sample analyte loading is exactly the same as previously described. The general workflow of the sample loading is presented in **Table 2**.

Table 2. Sample Analyte Load Workflow for Finnpipette Novus i Electronic 12-Channel Pipette using a speed setting of 5 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Analytical Sample	150	125	~400	~ 19 minutes
3	Wash Buffer	200	175	10	20 seconds
4	Water	200	150	10	20 seconds
5	Water	200	150	10	20 seconds

Note: The general workflow provide is to serve as a template for application. The rinse steps, number of cycle iterations, speed setting, etc. can all be tailored to the end user's application and is highly recommended for optimal assay performance.

22. Rinse the biotinylated-antibody-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer to remove non-specifically bound proteins. Select the WASH function on the Finnpipette Novus i Electronic 12-Channel Pipette and immerse the tips into the wash buffer pre-loaded into microtiter wells. Please adjust the speed, volume and number mixing cycles to match the values in **Table 2**. Again, press trigger button **once** to start each wash step.
23. Raise the MSIA Streptavidin D.A.R.T.'S above the Wash Buffer and press trigger once again to perform the BLOWOUT function.
24. Dilute the analyte-containing biological matrix (analytical sample) into a fixed volume of Dilution Buffer. Thoroughly mix the diluted sample via repeat inversion, vortexing or rotary platform. Dispense a measured aliquot of the analytical sample into either a well of a microtiter plate or a micro-centrifuge tube.
25. Load the biotinylated-antibody loaded MSIA Streptavidin D.A.R.T.'S with analytical sample. Select CAPTURE with OK (right selection key). Please adjust the speed, volume and mixing cycles to match the values in **Table 2** by pressing the edit button.
26. Lower the biotinylated-antibody-loaded MSIA Streptavidin D.A.R.T.'S into the analytical sample, again ensuring the distal ends are not pressed against the bottom of the microtiter wells. Press trigger button to start the cycling.

27. After completing CAPTURE step, lift MSIA Streptavidin D.A.R.T.'S above the samples and press trigger to BLOWOUT the remaining liquid from the tips.
28. Once the biotinylated-antibody MSIA Streptavidin D.A.R.T.'S are loaded with target analyte, they are ready for subsequent washes, elution, and any post elution treatment (if necessary). These steps are specific for the application and the analytical detection system that is selected by end user.
29. Select the WASH function with OK. Please adjust the speed, volume and number mixing cycles to match the values in **Table 2**. Again, press trigger button **once** to start each wash step.
30. After completing WASH step, lift MSIA Streptavidin D.A.R.T.'S above the Wash Buffer and press trigger to BLOWOUT the remaining liquid from the tips.
31. Repeat Step 30 & 31 for each of the water washes.
32. Transfer the eluent directly from the MSIA Streptavidin D.A.R.T.'S to the container from which the eluent will be further processed or analyzed from. This avoids loss of the eluent during the transfer, which may significantly impact analyte detection.
    Quick Tip: For LC-MS systems equipped with an autosampler capable of loading from microtiter plates, we recommend eluting into an AB1300 PCR plate for these purposes.
33. Fill the wells of an AB1300 plate, or alternative vial, with >50 uL elution solvent. An elution solvent must sufficiently disrupt the antibody/antigen complex. Some examples of elution solvents include acid (0.2-1% trifluoroacetic acid or formic acid) in 0-40% acetonitrile or isopropanol, base buffer (ammonium carbonate or Tris buffer pH 9-11) in 0-40% organic (acetonitrile, isopropanol), and denaturants (2-6M guanidine hydrochloride, 6-8M Urea). Care should be taken to not use an elution solvent that causes the captured analyte to precipitate within the MSIA pipette tip. To reduce loss of the eluted analyte to absorption to plastic or glassware, a blocking agent (e.g., 15 mg/L glucagon, adrenocorticotropic hormone (ACTH), or bovine serum albumin (BSA) in the elution solvent) may be used. Care should be taken in selecting a blocking agent that does not negatively affect downstream processes or detection of the targeted analyte.
34. Position the MSIA Streptavidin D.A.R.T.'S low enough into the wells containing a volume (10µL – 200µL) of the elution solvent can be pipetted without restriction in flow of the solvent through the pipette tips. Select the ELUTE function with OK. Depending on the affinity of the antibody for its antigen and the strength of the elution solvent, elution of the analyte from the MSIA pipette tip may require 30 seconds to ≥30 minutes.
35. Press trigger button to start elution step. After completing ELUTE step, lift MSIA Streptavidin D.A.R.T.'S above the samples and press trigger to BLOWOUT function.
36. Analyze protein biomarker with your selected detection instrumentation (e.g., mass spectrometer).

E. Forward MSIA Streptavidin Quick Start Protocol

Detailed instructions are in Sections C & D. *The settings provided are a general guideline and may require further optimization (increased or decreased) by the end-user.*

Quick Tip: Dedicate row(s) or column(s) of Wash Buffer(s) and Water in the microtiter plate for each wash step the assay.

Quick Tip: When positioning the tips, ensure the distal ends of the tips are placed just above the bottom of microtiter well and below the liquid's level. Please note pressing against the bottom of the microtiter wells will restrict the flow.

1. Dilute the biotinylated antibody with the Dilution Buffer for a final concentration range of 0.02 – 0.1 mg/mL. Add 125 µL of this solution into each well of the microtiter plate.
2. Load a well of Wash Buffer (200 µL) for each MSIA Streptavidin D.A.R.T.'S that will be used.
3. WASH- Pre-rinse the tips with Wash Buffer prior to biotinylated antibody loading (**Table 1, Step 1)**.

Table 1. Biotinylated Antibody Loading/Workflow for Finnpipette Novus i Electronic 12-Channel Pipette using a speed setting of 8 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Biotinylated Ab Solution	125	100	~400	14 minutes
3	Wash Buffer	200	175	10	20 seconds

4. BLOWOUT- Raise the Finnpipette Novus i Electronic 12-Channel Pipette so that the ends of the MSIA Streptavidin D.A.R.T.'S are above the liquid level, yet still remaining within the wells of the microtiter plate. Depress the trigger once to dispense any remaining liquid.
5. CAPTURE- Load MSIA Streptavidin D.A.R.T.'S with biotinylated antibody, then BLOWOUT (**Table 1, Step 2**).
6. WASH- Rinse the biotinylated-antibody-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer, then BLOWOUT (**Table 1, Step 3**).
7. Dilute analytical sample with the Dilution Buffer and internal reference standard (if applicable). Mix completely by repeat inversion, vortexing or rotary platform.
8. Dispense an aliquot of the analytical sample into an individual well of a microtiter plate or a micro-centrifuge tube.

Table 2. Sample Analyte Load Workflow for Finnpipette Novus i Electronic 12-Channel Pipette using a speed setting of 5 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Analytical Sample	150	125	~400	~ 19 minutes
3	Wash Buffer	200	175	10	20 seconds
4	Water	200	150	10	20 seconds
5	Water	200	150	10	20 seconds

9. WASH- Rinse the biotinylated-antibody-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer. BLOWOUT (**Table 2, Step 1).**
10. CAPTURE- Load the target analyte from the analytical sample, followed by BLOWOUT (**Table 2**, **Step 2**).

11. WASH- Wash the MSIA Streptavidin D.A.R.T.'S with Wash Buffer, followed by BLOWOUT (**Table 2, Step 3**).
12. WASH- Rinse with water to remove non-specific binding, followed by BLOWOUT (**Table 2, Steps 4-5)**.
13. Submerge the MSIA Streptavidin D.A.R.T.'S in a volume (10µL – 200µL) of the elution solvent in a low-bind plastic well or tube. Iteratively pipet the elution solvent to disrupt the antibody/antigen complex complex. Elution of the analyte from the MSIA Streptavidin D.A.R.T.'S may require 30 seconds to ≥30 minutes.

Reverse MSIA Streptavidin Protocol: Biotinylated Antigen Loading for Antibody extraction with Finnpipette Novus i Electronic 12-Channel Pipette.

Note: The following information provided in this procedure describes the use of the MSIA Streptavidin D.A.R.T.'S in the semi-automated Finnpipette Novus i Electronic 12-Channel Pipette bench top/mobile platform format. The steps described can easily be translated to a fully automated Versette robotic platform (please contact local MSIA support for more details). These procedures are intended for **general guidance only** and may require further optimization by the end-user.

F. Additional Materials Required

- Finnpipette Novus i Electronic Multichannel Pipette and Adjustable Pipette Stand (See Ordering Information)
- Assortment of Finnpipette*F1 Adjustable-Volume Pipettes
- Assortment of Finntip*Flex* Pipette Tips
- 96-well polypropylene microplates, 0.5mL, 1mL, or 2mL (Thermo Scientific Product No. 267334, 260251, 278743). Please note, we highly recommend using low-bind plastic ware to ensure efficient performance of the assay and prevent loss of analyte due to adsorption to the plastics.
- Fisher Chemical Optima LC/MS Water (Fisher Scientific Product No. W6-4).
- Dilution/Wash Buffer: Phosphate-buffered saline, pH 7.2 (PBS, Thermo Scientific Product No. 28372)

G. Alternate Equipment

- Versette Liquid Handling Platform (See Ordering Information)

H. Protocol Considerations

Biotinylated Antigen Loading

- The biotinylated antigen used for enrichment with this product should have sufficient affinity for the target antibody to ensure efficient capture of the analyte (antibody) of interest.
- Diluent/Wash Buffer can be prepared in bulk and stored at 4 °C until use.
- Buffers must be warmed to room temperature (25 °C) prior to use for efficient binding.
- The prepared biotinylated antigen solution used in the loading phase should be thoroughly mixed in the Dilution Buffer by repeat inversion or using a rotating platform. **Aggressive agitation of antigen solution will denature the protein and may result in loss of biotinylated antigen function**.
- At a minimum a single well of biotinylated antigen solution is necessary to perform each Streptavidin MSIA D.A.R.T.'S loading and analysis.
- Generally a row or column of the microtiter plate is assessed for assay development, thus allowing up to 8 (per column) or 12 (per row) samples to be addressed in parallel with the Finnpipette Novus i Electronic 12-Channel Pipette.

Sample Analyte Loading

- *Sample volume and concentration may be modified according to the end-user preference*. The sample dilution and volume are dictated by the biological matrix selected and the concentration of the analyte being targeted. If the analytical sample amount required exceeds the volume of the microtiter plate or micro-centrifuge tubes described in this protocol, then appropriately sized receptacles may be substituted, such as deep well microtiter plates.
- *Cycle volume and number of cycle iterations may be modified according to the end-user preference*. Studies should be performed by end-user to ensure optimal extraction/purification of the end-user's targeted analyte by the biotinylated-antigen loaded MSIA Streptavidin D.A.R.T.'S.
- Thaw and/or warm samples to at least room temperature before preparation. A 37 °C water bath may be employed to expedite this process and ensure consistent sample temperature.
  Quick Tip: Centrifuge raw samples prior to aliquot distribution to ensure the removal of any particulates or debris that may be present, which may clog the distal tip microcolumn.
    - Additional substances such as detergents, chaotropic agents or protein carries, e.g., Tween-20 Detergent (Thermo Scientific Product No. TA-125-TW) or Bovine Serum Albumin (10g/L concentration) can be added to each analytical sample to help prevent the antigen from adsorbing onto the plastic walls of the microtiter plate. These are suggested additions and the use of detergents can also reduce the ability of the antibody to bind its antigen, but ultimately specific concentrations and substances can be modified by the end user per their specific requirements. **Note: Do not use Tween-20 in Q-Exactive or other trap-based instrument applications as this polymeric detergent will potentially contaminant the instrument.**
- Use the Dilution Buffer to serially dilute the analytical sample. Thoroughly mix prior to the next dilution.
- An appropriate internal reference standard (IRS) may be added at a known concentration to the biological matrix (analytical sample) prior to antibody loading onto the MSIA Streptavidin D.A.R.T.'S. Generally, select an IRS with the same epitope as the endogenous antibody and that has a mass shift from the endogenous form. Ultimately, the IRS is application specific, and the user should empirically determine the appropriate concentration and standard. When the IRS is added during the CAPTURE step, it can be used to normalize losses throughout the sample-prep process.
- An elution solvent must sufficiently disrupt the antibody/antigen complex. Some examples of elution solvents include acid (0.2-1% trifluoroacetic acid or formic acid) in 0-40% acetonitrile or isopropanol, base buffer (ammonium carbonate or Tris buffer pH 9-11) in 0-40% organic (acetonitrile, isopropanol), and denaturants (2-6M guanidine hydrochloride, 6-8M Urea). Care should be taken to not use an elution solvent that causes the captured analyte to precipitate within the MSIA Streptavidin D.A.R.T.'S.
  Quick Tip: To reduce loss of the eluted analyte to absorption to plastic or glassware, a blocking agent (e. g., 15 mg/L glucagon, adrenocorticotropic hormone (ACTH) or bovine serum albumin (BSA) in the elution solvent) may be used. Care should be taken in selecting a blocking agent that does not negatively affect downstream processes or detection of the targeted analyte.
- Transfer the eluent directly from the MSIA Streptavidin D.A.R.T.'S to the container that will be used use for further processing or analysis to avoid loss of the eluent.

I. Reverse MSIA Streptavidin Protocol

1. Dilute the biotinylated antigen with the Dilution Buffer for a final concentration range of 0.02 – 0. 1 mg/mL. Add 125 µL of this solution into each well of the microtiter plate. At a minimum a single well of biotinylated antigen solution is necessary to perform each MSIA Streptavidin D.A.R.T.'S loading and analysis. Generally a row or column of the microtiter plate is assessed for assay development, thus allowing up to 8 (per column) or 12 (per row) samples to be addressed in parallel with the Finnpipette Novus i Electronic 12-Channel Pipette.
2. Load a well of Wash Buffer (200 µL) for each Thermo Scientific MSIA Streptavidin D.A.R.T.'S used.
   Quick Tip: Dedicate a row or column of Wash Buffer in the microtiter plate for assay development.
   Note: Larger volumes can be used with 2mL deep-well plates and by adjusting the pipette tip and stand heights.
3. If Finnpipette Novus i Electronic Adjustable Pipette Stand (sold separately) is used, snap on multichannel pipette bracket to Finnpipette Novus i Electronic 12-Channel Pipette.

4. Securely affix MSIA Streptavidin D.A.R.T.'S onto the Finnpipette Novus i Electronic 12-Channel Pipette nose cone. Mount the Finnpipette Novus i Electronic 12-Channel Pipette onto its stand, raise pipetter up from the deck. Insert microtiter plate onto the stand deck.
5. The general workflow for biotinylated antigen loading is presented in **Table 3**. The pipetting cycle iterations are performed by methodically aspirating and dispensing (standard pipette mixing action) using the listed cycle volumes provided. *The numbers of iterations provided are a general guideline and may require further optimization (increased or decreased) by the end-user.*

Table 3. Biotinylated Antigen Loading/Workflow for Finnpipette Novus i Electronic 12-Channel Pipette using a speed setting of 8 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Biotinylated Ag Solution	125	100	~400	14 minutes
3	Wash Buffer	200	175	10	20 seconds

6. Turn on Finnpipette Novus i Electronic 12-Channel Pipette by pressing the trigger button once.
7. Press Menu (left selection key) and scroll the function list with scroll key. Select MSIA function with OK (right selection key).
8. Position the sample plate under the pipette. Lower the MSIA Streptavidin D.A.R.T.'S into the Wash Buffer reservoirs using the slide vertical control knob on the stand. Ensure that the open end remains completely immersed in the volume of liquid during each step of the workflow to maintain proper sample and wash flow through the MSIA Streptavidin D.A.R.T.'S. Failure to do so will result in aspiration of air and inconsistency in tip treatment. Also, do not press the MSIA Streptavidin D.A.R.T.'S into the bottom of the liquid holding vesicle as this will restrict flow.
9. Pre-rinse streptavidin MSIA Streptavidin D.A.R.T.'S with Wash Buffer prior to loading with biotinylated antigen. Select the WASH function on the Finnpipette Novus i Electronic 12-Channel Pipette. The WASH submenu appears with preferred default values for the three variables of SPEED, VOLUME and number of CYCLES. Please adjust the speed, volume and mixing cycles to match the values in **Table 3** by pressing the edit button.
   Quick Tip: Nine PROGRAM submenus under the MSIA menu can be setup with user defined values and saved with preferred SPEED, VOLUME and CYCLE numbers using the pipette scroll key and selection keys.
   Quick Tip: Any program may be interrupted by selecting CANCEL (left selection key), which will stop the run.
10. Press the trigger button **once**, located on the back of the pipette handle, to begin rinsing of the tips with wash buffer. Immediately, the Finnpipette Novus i Electronic 12-Channel Pipette will begin mixing and the cycle numbers will start counting down. When finished, the Finnpipette Novus i Electronic 12-Channel Pipette will indicate completion of the WASH step by requiring the user to depress the trigger to perform the "BLOWOUT" function.
11. To BLOWOUT, raise the Finnpipette Novus i Electronic 12-Channel Pipette so that the distal ends of the MSIA Streptavidin D.A.R.T.'S are above the liquid level, yet still remaining within the wells of the microtiter plate. Depress the trigger once to allow the Finnpipette Novus i Electronic 12-Channel Pipette to dispense any remaining liquid from the tips.
12. Load the MSIA Streptavidin D.A.R.T.'S with biotinylated antigen. Scroll the function list and select CAPTURE with OK (right selection key). The CAPTURE submenu appears with preferred default values for the three variables of SPEED, VOLUME and number of CYCLES. Please adjust the speed, volume and mixing cycles to match the values in **Table 3** by pressing the edit button.
13. Lower the MSIA Streptavidin D.A.R.T.'S into the biotinylated antigen solution.
14. Press trigger button; cycling will start and the cycle number will count-down.

15. Lift the MSIA Streptavidin D.A.R.T.'S above the samples and press trigger to BLOWOUT the remaining liquid from the tips.
16. Rinse the biotinylated-antigen-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer. Select the WASH function on the Finnpipette Novus i Electronic 12-Channel Pipette, immerse the tips into the wash buffer pre-loaded into microtiter wells, and then press the trigger to begin rinsing of the tips with wash buffer. Please adjust the speed, volume and mixing cycles to match the values in **Table 3** by pressing the edit button.
17. Raise the MSIA Streptavidin D.A.R.T.'S above the Wash Buffer and press trigger once again to perform the BLOWOUT function.
18. Once the MSIA Streptavidin D.A.R.T.'S are loaded with biotinylated antigen, they can be immediately used for target analyte purification.
19. Dilute the analyte-containing biological matrix with a known volume of Dilution Buffer and internal reference standard (if applicable). Mix the diluted sample thoroughly via repeat inversion, vortexing or rotary platform. Dispense a measured aliquot of the analytical sample into either an individual well of a microtiter plate or a micro-centrifuge tube.
20. Load a well of Wash Buffer (200 µL) and corresponding wells of water (200 µL per) for each MSIA Streptavidin D.A.R.T.'S used. Washing removes non-specifically bound impurities.
    Quick Tip: Dedicate a row or column of Wash Buffer and Water in the microtiter plate for assay development.
    Note: Larger volumes can be used with 2mL deep-well plates and by adjusting the pipette tip and stand heights.
21. The mechanical application of the MSIA Streptavidin D.A.R.T.'S in sample analyte loading is exactly the same as previously described. The general workflow of the sample loading is presented in **Table 4**.

Table 4. Sample Analyte Load Workflow for Finnpipette Novus i Electronic 12-Channel Pipette using a speed setting of 5 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Analytical Sample	150	125	~400	19 minutes
3	Wash Buffer	200	175	10	20 seconds
4	Water	200	150	10	20 seconds
5	Water	200	150	10	20 seconds

Note: The general workflow provide is to serve as a template for application. The rinse steps, number of cycle iterations, etc. can all be tailored to the end user's application.

22. Rinse the biotinylated-antigen-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer to remove non-specifically bound proteins. Select the WASH function on the Finnpipette Novus i Electronic 12-Channel Pipette and immerse the tips into the wash buffer pre-loaded into microtiter wells. Please adjust the speed, volume and number mixing cycles to match the values in **Table 4**. Again, press trigger button **once** to start each wash step.
23. Raise the MSIA Streptavidin D.A.R.T.'S above the Wash Buffer and press trigger once again to perform the BLOWOUT function.
24. Dilute the analyte-containing biological matrix (analytical sample) into a fixed volume of Dilution Buffer. Thoroughly mix the diluted sample via repeat inversion, vortexing or rotary platform. Dispense a measured aliquot of the analytical sample into either a well of a microtiter plate or a micro-centrifuge tube.
25. Load the biotinylated-antigen-loaded MSIA Streptavidin D.A.R.T.'S with analytical sample. Select CAPTURE with OK (right selection key). Please adjust the speed, volume and mixing cycles to match the values in **Table 4** by pressing the edit button.
26. Lower the biotinylated-antigen-loaded-MSIA Streptavidin D.A.R.T.'S into the analytical sample, again ensuring the distal ends are not pressed against the bottom of the microtiter wells. Press trigger button to start the cycling.

27. After completing CAPTURE step, lift MSIA Streptavidin D.A.R.T.'S above the samples and press trigger to BLOWOUT the remaining liquid from the tips.
28. Once the biotinylated-antigen-MSIA-Streptavidin-D.A.R.T.'S are loaded with target analyte, they are ready for subsequent washes, elution, and any post elution treatment (if necessary). These steps are specific for the application and the analytical detection system that is selected by end user.
29. Select the WASH function with OK. Please adjust the speed, volume and number mixing cycles to match the values in **Table 4**. Again, press trigger button **once** to start each wash step.
30. After completing WASH step, lift MSIA Streptavidin D.A.R.T.'S above the samples and press trigger to BLOWOUT the remaining liquid from the tips.
31. Repeat Step 29 & 30 for each of the water washes.
32. Transfer the eluent directly from the MSIA Streptavidin D.A.R.T.'S to the container from which the eluent will be further processed or analyzed from. This avoids loss of the eluent during the transfer, which may significantly impact analyte detection.
    Quick Tip: For LC-MS systems equipped with an autosampler capable of loading from mirotiter plates, we recommend eluting into an AB1300 PCR plate for these purposes.
33. Fill the wells of an AB1300 plate, or alternative vials, with >50 µL elution solvent. An elution solvent must sufficiently disrupt the antibody/antigen complex. Some examples of elution solvents include acid (0.2-1% trifluoroacetic acid or formic acid) in 0-40% acetonitrile or isopropanol, base buffer (ammonium carbonate or Tris buffer pH 9-11) in 0-40% organic (acetonitrile, isopropanol), and denaturants (2-6M guanidine hydrochloride, 6-8M Urea). Care should be taken to not use an elution solvent that causes the captured analyte to precipitate within the MSIA tip. To reduce loss of the eluted analyte to absorption to plastic or glassware, a blocking agent (e. g. , 15 mg/L glucagon, adrenocorticotropic hormone (ACTH), or bovine serum albumin (BSA) in the elution solvent) may be used. Care should be taken in selecting a blocking agent that does not negatively affect downstream processes or detection of the targeted analyte.
34. Position the MSIA Streptavidin D.A.R.T.'S low enough into the wells containing a volume (10µL – 200µL) of the elution solvent can be pipetted without restriction in flow of the solvent through the tips. Select the ELUTE function with OK. Depending on the affinity of the antibody for its antigen and the strength of the elution solvent, elution of the analyte from the MSIA tip may require 30 seconds to ≥30 minutes.
35. Press trigger button to start elution step. After completing ELUTE step, lift MSIA Streptavidin D.A.R.T.'S above the samples and press trigger to BLOWOUT function.
36. Analyze protein biomarker with your selected detection instrumentation (e.g., mass spectrometer).

J. Reverse Streptavidin MSIA Quick Start Protocol

Detailed instructions are in Sections H & I. *The settings provided are a general guideline and may require further optimization (increased or decreased) by the end-user.*

Quick Tip: Dedicate row(s) or column(s) of Wash Buffer and Water in the microtiter plate for each wash step the assay.
Quick Tip: When positioning the tips, ensure the distal ends of the tips are placed just above the bottom of microtiter well and below the liquid's level. Please note pressing against the bottom of the microtiter wells will restrict the flow.

1. Dilute the biotinylated antigen with the Dilution Buffer for a final concentration range of 0.02 – 0.1 mg/mL. Add 125 µL of this solution into each well of the microtiter plate.
2. Load a well of Wash Buffer (200 µL) for each MSIA Streptavidin D.A.R.T.'S that will be used.
3. WASH- Pre-rinse the MSIA Streptavidin D.A.R.T.'S with Wash Buffer prior to biotinylated antigen loading (**Table 3, Step 1)**.

Table 3. Biotinylated Antigen Loading/Workflow for MSIA Streptavidin D.A.R.T.'S using a speed setting of 8 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Biotinylated Ag Solution	125	100	~400	14 minutes
3	Wash Buffer	200	175	10	20 seconds

4. BLOWOUT- Raise the MSIA Streptavidin D.A.R.T.'S so that the ends of the MSIA Streptavidin D.A.R.T.'S are above the liquid level, yet remaining within the wells of the microtiter plate. Depress the trigger once to dispense any remaining liquid.
5. CAPTURE- Load MSIA Streptavidin D.A.R.T.'S with biotinylated antigen, then BLOWOUT (**Table 3, Step 2**).
6. WASH- Rinse the biotinylated-antigen-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer, then BLOWOUT (**Table 3, Step 3**).
7. Dilute analytical sample with the Dilution Buffer and internal reference standard (if applicable). Mix completely by repeat inversion, vortexing or rotary platform.
8. Dispense an aliquot of the analytical sample into an individual well of a microtiter plate or a micro-centrifuge tube.

Table 4. Sample Analyte Load Workflow for MSIA Streptavidin D.A.R.T.'S using a speed setting of 5 for the mixing cycles:

Steps	Description	Microtiter plate Volume (µL)	Mixing Cycle Volume (µL)	No. of Mixing Cycle Iterations	Approx. Allotted time per step
1	Wash Buffer	200	175	10	20 seconds
2	Analytical Sample	150	125	~400	19 minutes
3	Wash Buffer	200	175	10	20 seconds
4	Water	200	150	10	20 seconds
5	Water	200	150	10	20 seconds

9. WASH- Rinse the biotinylated-antigen-loaded MSIA Streptavidin D.A.R.T.'S with Wash Buffer, then BLOWOUT (**Table 4, Step 1**).
10. CAPTURE – Load the target analyte from the analytical sample, followed by BLOWOUT (**Table 4**, **Step 2**).
11. WASH – Wash the MSIA Streptavidin D.A.R.T.'S with Wash Buffer, followed by BLOWOUT (**Table 4, Step 3**).
12. WASH – Rinse with water to remove non-specific binding, followed by BLOWOUT (**Table 4, Steps 4-5**).
13. ELUTE- Iteratively pipette the elution solvent (10µL – 200µL) to disrupt the antibody/antigen complex. Elution of the analyte from the MSIA Streptavidin D.A.R.T.'S may require 30 seconds to ≥ 30 minutes or more.

Explanation for Variation in pipetting cycles and speed:

The rationale for identifying the speed (rate) and the number of pipetting cycles (i.e., the amount of time for incubating the MSIA Streptavidin D.A.R.T.'S with the antibody or samples) is based on the law of mass action. There are two

aspects that describe this law: 1) concentrations of reactants (antibody, antigen, streptavidin/biotin etc.) and products; and 2) the rate constant of the reaction.

With respect to aspect 1 of the law of mass action (concentrations), lower concentrations of the reactants react slower. In the MSIA enrichment protocol using MSIA Streptavidin D.A.R.T.'S slower reactions necessitates longer incubation times during analyte capture. Longer incubation times are accomplished by increasing the number of pipetting iterations and/or slowing down the pipetting rate. Table A2 provides guidance to the rate and number of iterations for pipetting using the MSIA Streptavidin D.A.R.T.'S when addressing samples consisting of different concentrations of the analyte.

The second aspect of the law of mass action is the reaction rate. With respect to streptavidin, the affinity constant is stable and strong in the immobilization of the biotinylated molecule due to the fact of the strong affinity bond between streptavidin and biotin. But this law has significance when it comes to the capture of the analyte of interest. For an antibody/antigen system this refers to the affinity constant that describes the antibody's affinity for its antigen. Therefore, a higher affinity constant (i.e., stronger attraction) will result in a faster formation of the complex. In the MSIA Streptavidin D.A.R.T.'S workflow, an antibody with a high affinity constant to its analyte will require less incubation time. Therefore, higher speed settings and fewer pipetting iterations. However, for lower affinity constants slower speed setting and a greater number of iterations will be required. The goal is to match the speed and iterations of the pipetting to the kinetics of the reaction.

As recommended in the instructions above, only antibodies with >1:8,000 working dilutions for EIA or ELISA are recommended for use with MSIA Streptavidin/Biotinylated Ligand D.A.R.T.'S. Antibodies with lower affinities do not have a sufficient affinity and are likely to result in poor enrichment of their antigen.

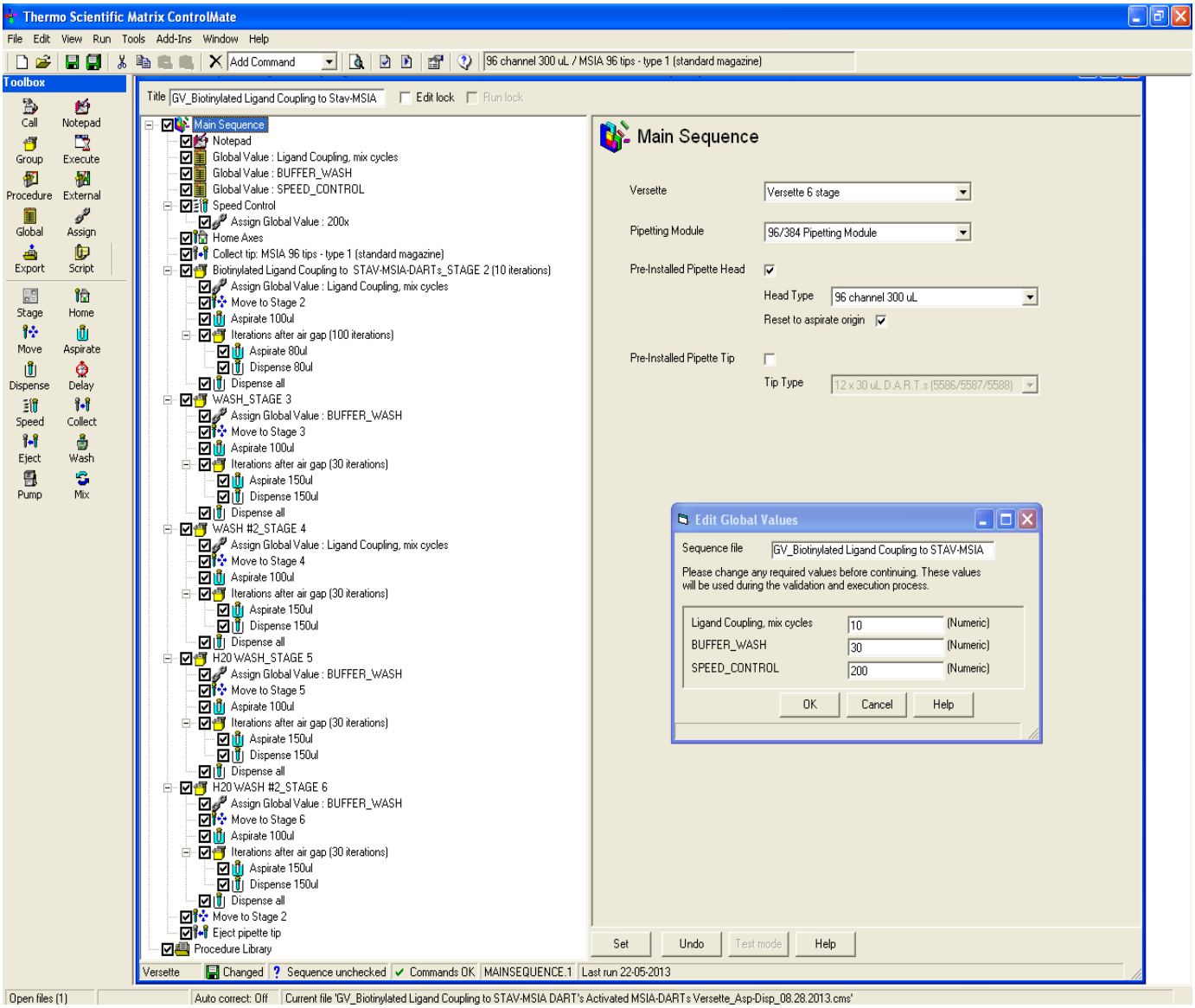
Analyte Concentration	Sample Volume	Pipetting iterations and rate
≥ µg/mL	≤ 100µL	50 – 400 cycles, Novus i speed 5 – 8
ng/mL	10 - 500µL	100 – 1500 cycles, Novus i speed 2 – 6
≤ pg/mL	≥ 500µL	500 – 2000 cycles, Novus i speed 2 – 5

In the examples outlined below Biotinylated IgG and Biotinylated Bovine Serum Albumin are used as examples, respectively, for the forward and reverse aspects of the MSIA Streptavidin D.A.R.T.'S system using the Robotic Versette platform. Both biotinylated substances were immobilized to the Streptavidin surface in phosphate buffered saline pH 7. 4. The capacities of Streptavidin to capture these biotinylated substances were analyzed by the use of a Micro Bicinchoninic Acid (µBCA) protein kit.

MSIA D.A.R.T. Surface	pH range (optimal)	Compatible buffers
Streptavidin	pH 7-7. 6 (pH 7. 4)	Phosphate Buffered Saline (PBS), Hepes Buffered Saline (HBS), Tris Buffered Saline (TBS)

Example protocol of Streptavidin/Biotin Ligand coupling at room temperature using Versette Automated Liquid Handler

MSIA Streptavidin D.A.R.T.'S -100 µl of each biotinylated substance was used, i.e., **400** repetitions using global values (GV).



Additional Information

Visit the www.thermoscientific.com/msia for additional information and application notes relating to this product.

Visit www.thermoscientific.com/pierce for information specifically related to Streptavidin.

Product stable for 18 months from date of manufacture when handled and stored according to Manufacturer instructions, see details under **Warranty**.

The most current versions of all MSIA product instructions are available at www.thermoscientific.com/msia.

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.*

Warranty: Sellers warrants that the Products will operate or perform substantially in conformance with Seller's published specifications and be free from defects in material and workmanship, when subjected to normal, proper and intended usage by properly trained personnel, for the period of time set forth in the product documentation, published specifications or page insert. If a period of time is not specified in Seller's product documentation, published specifications or package inserts, the warranty period shall be one (1) year from the date of shipment to Buyer for equipment and ninety (90) days for all other products (the "Warranty Period"). Seller agrees the Warranty Period, to repair or replace at Seller's option, defective Products so as to cause the same to operate in substantial conformance with said published specifications, provided that Buyer shall (a) promptly notify Seller in writing upon the discovery of any defect, which notice shall include the product model and serial number (if applicable) and details of the warranty claim, and (b) after Seller's review. Seller will provide Buyer with service data and/or a Return Material Authorization ("RMA"), which may include biohazard decontamination procedures and other product-specific handling instructions, then, if applicable, Buyer may return the effective Products to Seller with all costs prepaid by Buyer. Replacement parts may be new or refurbished at the election of Seller. All replaced parts shall become the property of Seller. Shipment to Buyer repaired or replacement Products shall be made in accordance with the Delivery provisions of the Seller's Term and Conditions of Sale. Consumables are expressly excluded this warranty. Notwithstanding the foregoing, Products supplied by Seller that are obtained by Seller from an original manufacturer or third party supplier are not warranted by Seller, but Seller agrees to assign to Buyer any warranty rights in such Product that Seller may have from the original manufacturer or third party supplier, to the extent such assignment is allowed by such original manufacturer or third party supplier. In no event shall Seller have any obligation to make repairs, replacements or corrections required, in whole or in part, as the result as (i)normal wear and tear, (ii)accident, disaster or event of force majeure, (iii)misuse, fault or negligence of or by Buyer, (iv)use of the Products in a manner for which they were not designed, (v)causes external to the Products such as, but not limited to, power failure or electrical power surges, (vi)improper storage and handling of the Products or (vii) use of the Products in combination with equipment or software not supplied by Seller. If Seller determines that Products for which Buyer has requested warranty services are not covered by the warranty hereunder. Buyer shall pay or reimburse Seller for all costs of investigating and responding to such request at Seller's then prevailing time and materials rates. If Seller provides repair services or replacement parts that are not covered by this warranty, Buyer shall pay Seller therefore at Seller's then prevailing time and materials rates. Any installation, maintenance, repair, service, relocations or alteration to or of, or other tampering with, the products preformed by any person or entity other than seller without sellers' prior written approval, or any use of replacement parts not supplied by seller, shall immediately void and cancel all warranties with respect to the affected products. The obligations created by this warranty statement to repair or replace a defective product shall be the sole remedy of buyer in the event of defective product except as expressly provided in this warranty statement, seller disclaims all other warranties, whether express or implied, oral or written, with respect to the products, including without limitations all implied warranties of merchantability or fitness for any particular purpose. Seller does not warrant that the products are error-free or will accomplish any particular result.

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Rev A