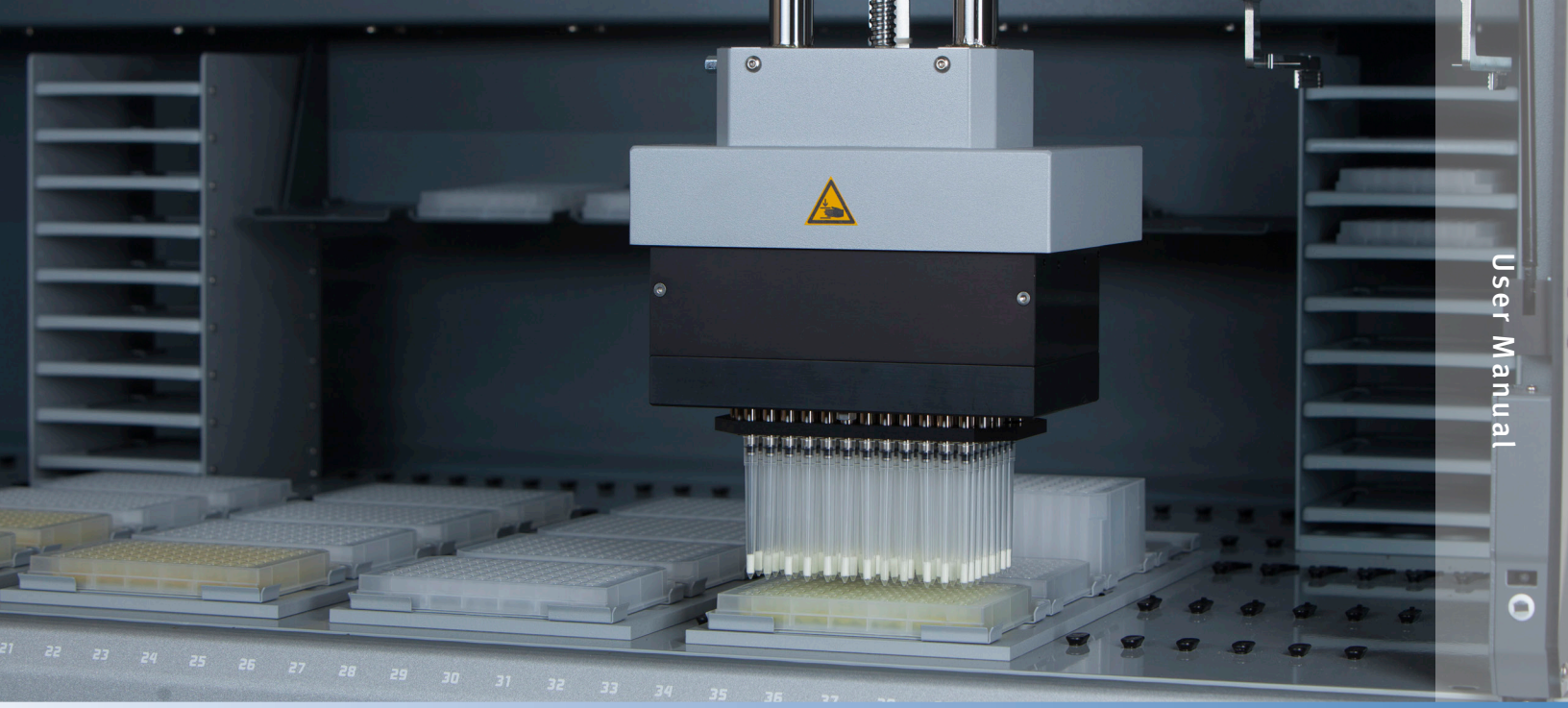


Protocol for the use of MSIA™ Streptavidin-EVO

Utilizing Thermo Scientific™ MSIA™ Streptavidin EVO Microcolumns, Tecan™ and Freedom EVO® 150 Liquid Handling Robotic Platform

992STR96

MSIA Streptavidin EVO microcolumns containing 1 rack of 96 units.

Thermo SCIENTIFIC

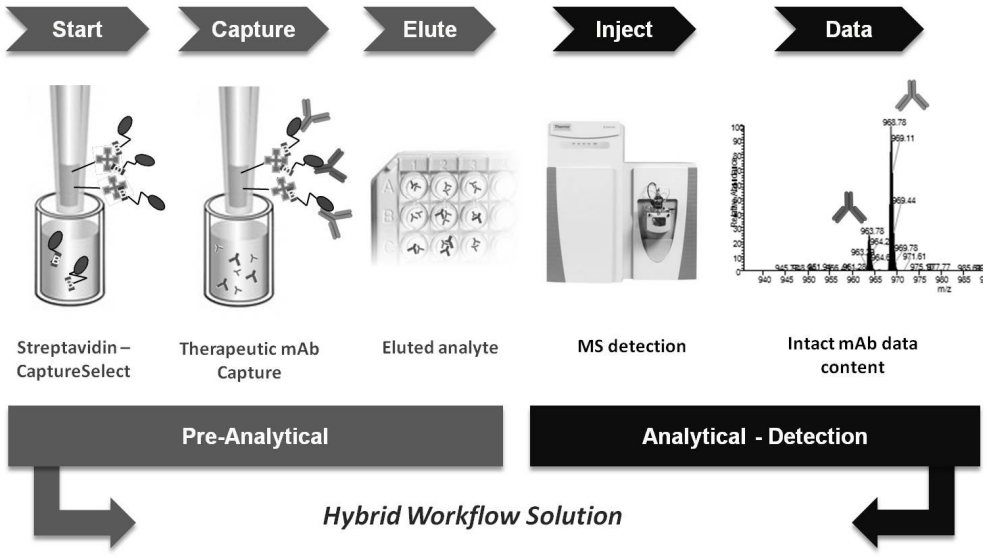
Table of Contents

Chapter 1

Introduction

The Mass Spectrometric Immunoassay (MSIA™) approach coupled with the Tecan Freedom EVO high throughput MCA-96 robotic platform delivers a fast, convenient and highly reproducible workflow for the analysis of target analytes with subsequent mass spectrometric detection. Thermo Scientific™ MSIA™ Streptavidin-EVO uses molecular trapping microcolumn technology embedded within a pipette housing covalently derivatized with recombinant streptavidin (53 kDa) that provides the end user with enhanced capability to customize the assay for their specific applications by using their own biotinylated ligands. Interrogation of a prepared biological sample by repetitive bi-directional pipetting (aspirating and dispensing using mix command) using MSIA Streptavidin EVO microcolumns loaded with a biotin conjugated ligand allows for increased efficiency of the capture of a targeted analyte. The microcolumns with the affinity captured analyte undergo a series of rinses followed by elution with an elution buffer. The ensuing eluates containing the target analyte are then ready to be analyzed by LC-MS. This hybrid bioanalytical workflow combines affinity capture with mass spectrometric detection enabling greater dynamics and specificity for both qualitative and quantitative analyses of protein targets such as protein biologics.

Graphic 1 – Ligand Binding—Mass Spectrometric Immunoassay Workflow



Chapter 2

Important Product Information

- Storage Conditions: MSIA Streptavidin EVO microcolumns should be stored at 4°C for up to the specified expiration date indicated on the product. This product should never be frozen.

- The MSIA Streptavidin EVO microcolumns are intended for single use only. The product is not intended for the transference or measurement of liquids.

- **Safety:** All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

- **Disposal:** This product may come in contact with biological materials during its use. Product should be treated in accordance with universal precautions established by the Centers for Disease Controls and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

- **Disclaimer:** This product is intended for research use only.

3

Chapter 3

MSIA Streptavidin-EVO Workflow Protocol

⚠ These instructions are intended to begin initial testing and optimization of a ligand binding MSIA workflow. While this protocol may be utilized as a starting point, it is recommended that the conditions of this protocol be individually optimized for each specific analyte and antibody combination.

Reference the following application note for an example of a MSIA Streptavidin-EVO workflow used to assay therapeutic monoclonal antibodies from rodent plasma: *A universal LB-MSIA workflow using Freedom Evo platform for the pre-clinical analysis of therapeutic antibodies of differing allotypes in rodent plasma.*

3A. Equipment and Materials Required

Equipment

- Tecan™ Freedom EVO® 150 Liquid Handling Robotic Platform equipped with a MCA96 head
- Set of Finnpipette™ F1 Adjustable-Volume Pipettes (Thermo Scientific™, Product No. 4700850) or equivalent

Materials for Derivatization of MSIA Streptavidin EVO Microcolumns with Biotin Conjugate Affinity Ligand

- MSIA Streptavidin-EVO (Thermo Scientific™, Product No. 992STR96)
- Biotinylated Affinity Ligand
- BupH™ Modified Dulbecco's Phosphate Buffered Saline Packs (PBS); 8mM sodium phosphate, 2mM potassium phosphate, 0.14M NaCl, 10mM KCl, pH 7.4. (Thermo Scientific, Product No. 28374)
- Nunc™ 96-Well Polypropylene Plates, 500µL (Thermo Scientific™, 267245)

Additional Materials for Sample Preparation and Capture

- Rodent Plasma or alternative 50g/L Bovine Serum Albumin in 10mM PBS
- Analyte
- **Recommended:** Internal Reference Standard that cross reacts with the affinity ligand
- Water, Optima™ LC/MS (Fisher Chemical, Product No. W6)

Additional Materials for Analyte Elution

- MSIA Elution Buffer

⚠ The MSIA Elution Buffer is a proprietary product recommended for all high throughput applications that reduces the loss in protein analyte due to its absorption to the plastics.

An alternative elution buffer option is to use 0.1% Zwittergent® (Calbiochem™) in 2% Formic Acid with 10% Methanol, however different from the MSIA elution buffer the alternative elution buffer does not fully prevent loss of protein analyte due to its absorption to the plastics, which increases with time. If utilizing the alternative elution buffer ensure the samples are loaded onto the LC-MS within one hour of eluting.

For any questions concerning appropriate elution solvents and the MSIA Elution Buffer please use the following contact information:

Technical literature is available at: www.thermoscientific.com/msia
Contact Support: +1 800 345 0206
Outside North America Toll Free: + 1 858 453 7551
Email: msia.info@thermofisher.com

3B. Tecan Freedom EVO MSIA Scripts

⚠ Before beginning the script generation, please note that the carrier template utilized by your Tecan Freedom EVO will likely vary from the templates represented below in Figures 3, 4, and 5. It is due to the unique setup of each Tecan Freedom EVO that pre-made scripts cannot be readily available.

For any questions concerning initial setup of the MSIA Streptavidin-EVO scripts on your Tecan EVO please contact customer support at:

Technical literature is available at: www.thermoscientific.com/msia
Contact Support: +1 800 345 0206
Outside North America Toll Free: + 1 858 453 7551
Email: msia.info@thermofisher.com

3B.1: Preparation of the Labware

The following default labware is utilized in the MSIA Streptavidin-EVO scripts presented in this manual and should be tested on each system for proper alignment prior to running the MSIA Streptavidin-EVO workflow.
In the Freedom EVOware, select the "Labware" tab of the Control Bar located on the left side of the Freedom EVOware window. Adjust the alignment as necessary for each individual Tecan Freedom EVO.

i. Microplates: **96 Well Microplate**

ii. Microplates: **96 Well Deepwell**

iii. Tips: **DiTi 500ul SBS MCA96** – Be sure that the MSIA Streptavidin EVO microcolumns are used for initial alignment and not basic DiTis.

Note: If the labware is not present, right click the labware list and from the drop down menu select "Show hidden". This will bring up the complete list of labware.

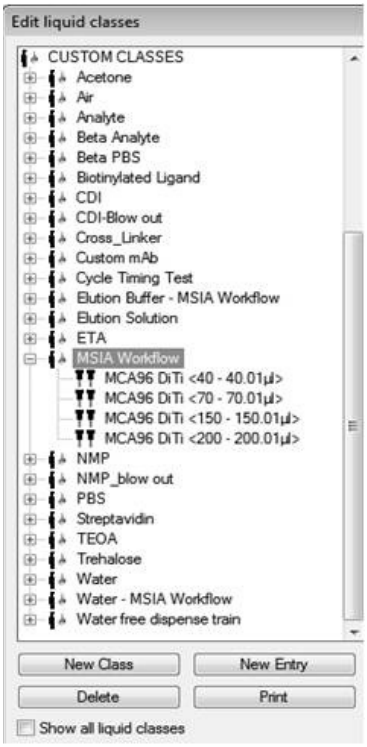


Figure 1 – MSIA Workflow, Liquid Class

3B.2: Preparation of the Liquid Class

A new liquid class should be created based off of the density of water in order to simplify the Freedom EVO script generation for the MSIA Streptavidin-EVO workflow. Refer to Table 1 and Figure 1 for the recommended settings to create the MSIA Workflow Liquid Class.

Table 1 – MSIA Workflow Liquid Class Entries

	Volume (µL)	Type	Asp/Disp Speed (µL/sec)	Air Gap (µL)
1	40-40.01	MCA96 DiTi	45	50
2	70-70.01	MCA96 DiTi	45	50
3	150-150.01	MCA96 DiTi	115	50
4	200-200.01	MCA96 DiTi	46	50

3B.3: Preparation of the Worktable Template

Figure 2 below shows an example worktable template used to run the MSIA Streptavidin-EVO scripts. The carriers used are 96-Well Plate carriers with 4 positions (Tecan part no. 30013061). While five carriers are shown in this template only two (n = 6 x 96-well positions) are necessary to perform the MSIA workflow presented in this instruction manual.

- Ensure that the necessary labware is allowed on the 96-well carriers being utilized: **96 Well Microplate, 96 Well Deepwell, DiTi 500ul SBS MCA96**

Figure 2–MSIA Workflow Worktable Template

3B.4: Preparation of the MSIA Streptavidin-EVO Biotinylated Ligand Coupling Script File

The MSIA Streptavidin-EVO Biotinylated Ligand Coupling Script will perform a series of aspiration and dispense cycles in order to facilitate the capture of the biotinylated affinity ligand by the MSIA Streptavidin EVO microcolumns. The affinity ligand capture will be supplemented by several rinses as outlined in Table 2. Figure 3 (pg. 13) shows the completed Biotinylated Ligand Capture Script that should be used as a reference for script generation.

Table 2–MSIA Biotinylated Ligand Coupling Overview

	Assay Step	Command	Labware	Liquid Class	Asp/Disp Volume (µL)	Asp/Disp Cycles	Asp/Disp Speed (µL/sec)
1	Pre-Rinse: PBS	Mix	96 Well Microplate	MSIA Workflow	150	10	115
2	Biot-Affinity Ligand Capture	**Loop/Mix**	96 Well Microplate	MSIA Workflow	70	**100 x 5***	45
3	Rinse 1: PBS	Mix	96 Well Microplate	MSIA Workflow	150	10	115
4	Rinse 2: PBS	Mix	96 Well Microplate	MSIA Workflow	150	10	115

*The Mix Command has a maximum setting of 100 cycles. A loop will be added to repeat the 100 cycles of the Biotinylated Affinity Ligand 5 times for a total of 500 cycles.

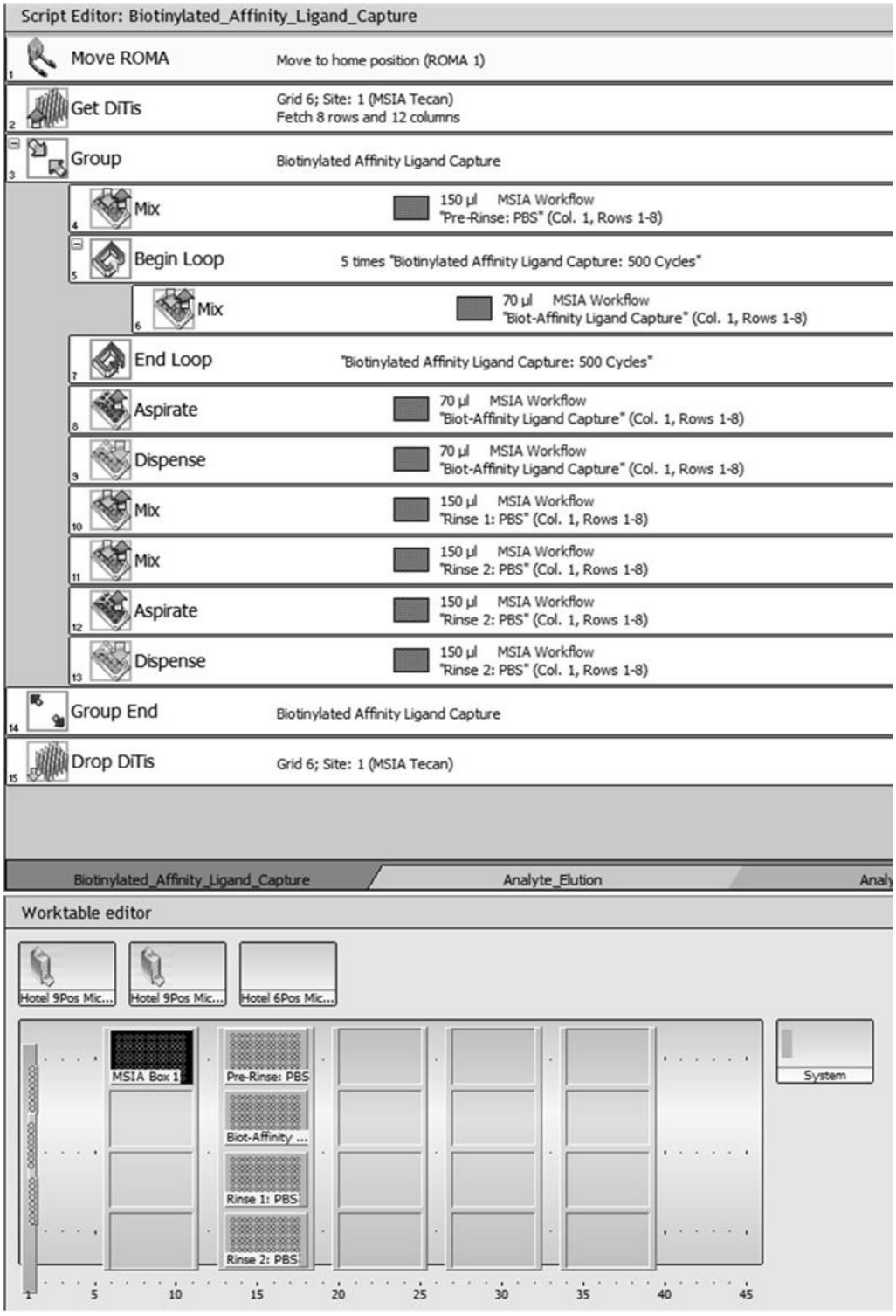
- **Command: Aspirate and Dispense (MCA96)** – The aspirate and dispense commands in the script (Reference Figure 3) are necessary to achieve a blowout, which is not intrinsic to the Mix Command. The blowout will expel the air gap in order to reduce the amount of liquid retained in the MSIA Streptavidin EVO microcolumns as the microcolumns transition from step to step.

Prepare the two aspirate and dispense commands within the Biotinylated Ligand Coupling script using the settings outlined in Table 3.

Table 3–Biotinylated Ligand Coupling Script Aspirate/Dispense Commands

	Linked Carrier Plate	Liquid Class	Volume (µL)
1	Biot-Affinity Ligand	MSIA Workflow	70
2	Rinse 2: PBS	MSIA Workflow	150

Figure 3–MSIA Streptavidin-EVO Biotinylated Ligand Coupling Completed Script Reference



3B.5: Preparation of the MSIA Streptavidin-EVO Analyte Capture Script File

The MSIA Streptavidin-EVO Analyte Capture Script will perform a series of aspiration and dispense cycles in order to facilitate the capture of the analyte from the sample by the MSIA Streptavidin EVO microcolumns. The analyte capture will be supplemented by several rinses as outlined in Table 4. Figure 4 (pg. 15) shows the completed Analyte Capture Script that should be used as a reference for script generation.

Table 4–MSIA Analyte Capture Overview

	Assay Step	Command	Labware	Liquid Class	Asp/Disp Volume (µL)	Asp/Disp Cycles	Asp/Disp Speed (µL/sec)
1	Sample Plate	Loop/Mix	96 Well Deepwell	MSIA Workflow	200	89 x 7*	46
2	Rinse 1: PBS	Mix	96 Well Microplate	MSIA Workflow	150	10x	115
3	Rinse 2: PBS	Mix	96 Well Microplate	MSIA Workflow	150	10x	115
4	Rinse 3: Water	Mix	96 Well Microplate	MSIA Workflow	150	10x	115
5	Rinse 4: Water	Mix	96 Well Microplate	MSIA Workflow	150	10x	115

*The Mix Command has a maximum setting of 100 cycles. A loop will be added to repeat the 89 cycles of the Analyte Capture 7 times for a total of 623 cycles.

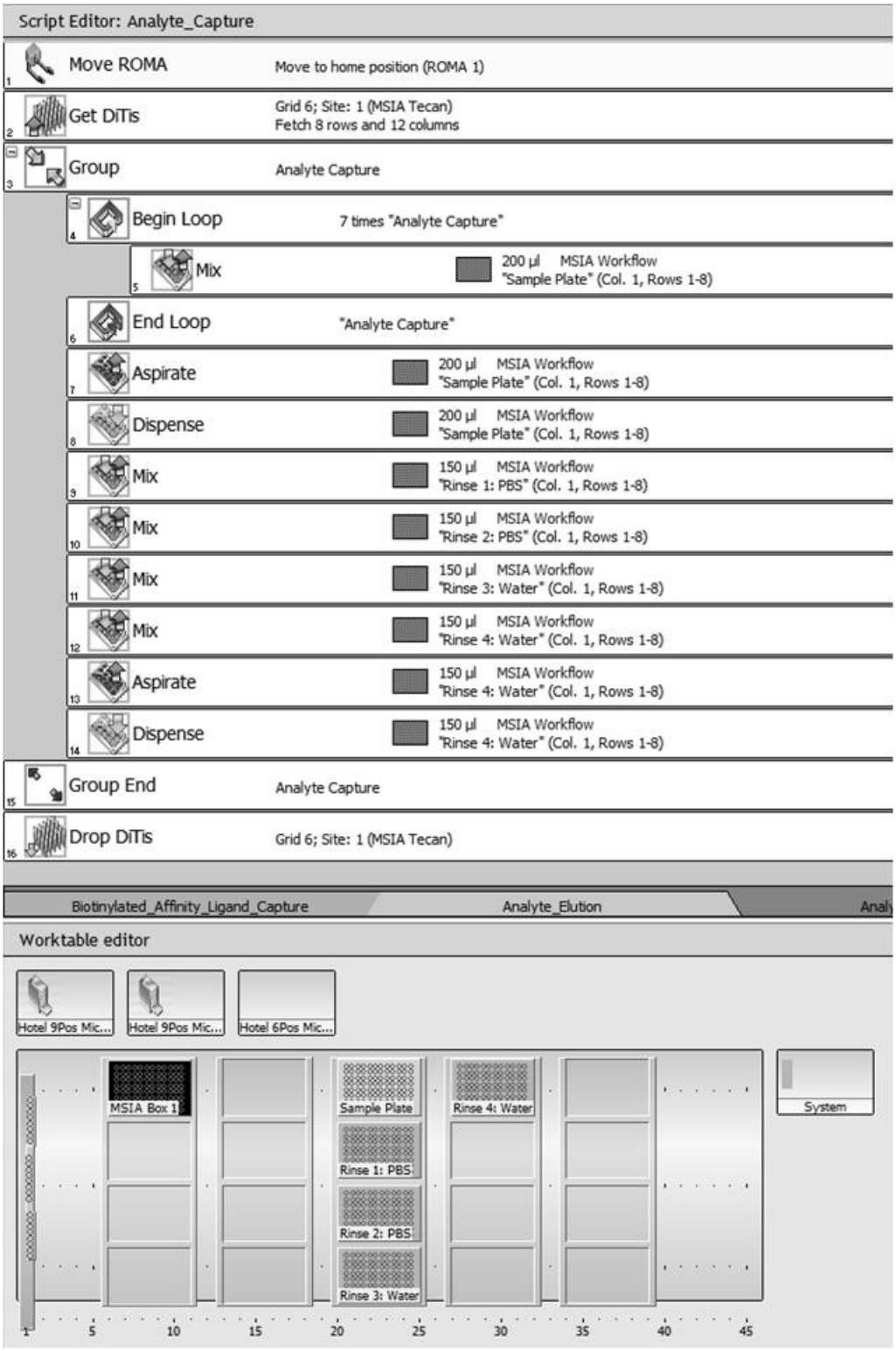
- **Command: Aspirate and Dispense (MCA96)** – The aspirate and dispense commands in the script (Reference Figure 4) are necessary to achieve a blowout, which is not intrinsic to the Mix Command. The blowout will expel the air gap in order to reduce the amount of liquid remaining in the MSIA Streptavidin EVO microcolumns as the microcolumns transition from step to step.

Prepare the two aspirate and dispense commands within the Analyte Capture script using the settings outlined in Table 5.

Table 5–Analyte Capture Script Aspirate/Dispense Commands

	Linked Carrier Plate	Liquid Class	Volume (µL)
1	Sample Plate	MSIA Workflow	200
2	Rinse 4: Water	MSIA Workflow	150

Figure 4—MSIA Streptavidin-EVO Analyte Capture Completed Script Reference



3B.6: Preparation of the MSIA Streptavidin-EVO Analyte Elution Script File

The MSIA Streptavidin-EVO Analyte Elution Script will perform a series of aspiration and dispense cycles in order to facilitate the elution of the analyte from the MSIA Streptavidin EVO microcolumns. The analyte elution is outlined in Table 6. Figure 5 (pg. 17) shows the completed Analyte Elution Script that should be used as a reference for script generation.

Table 6–MSIA Analyte Elution Overview

	Assay Step	Command	Labware	Liquid Class	Asp/Disp Volume (µL)	Asp/Disp Cycles	Asp/Disp Speed (µL/sec)
1	Analyte Elution	Mix	96 Well Microplate	MSIA Workflow	40	20x	48

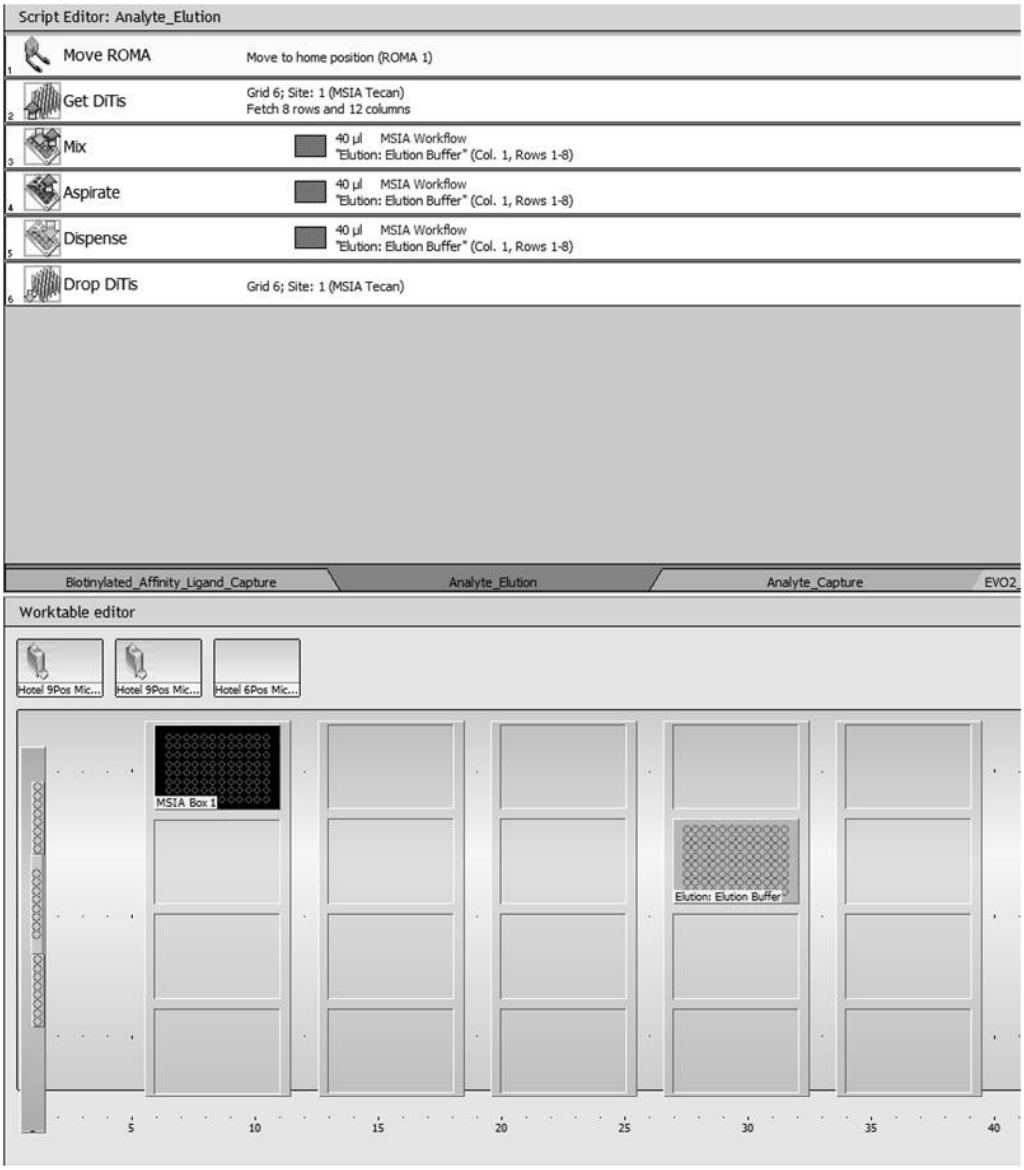
- **Command: Aspirate and Dispense (MCA96)** – The aspirate and dispense commands in the script (Reference Figure 5) are necessary to achieve a blowout, which is not intrinsic to the Mix Command. The blowout will expel the air gap in order to reduce the amount of liquid remaining in the MSIA Streptavidin EVO microcolumns as the microcolumns transition from step to step.

  Prepare the aspirate and dispense commands within the Analyte Elution script using the settings outlined in Table 7.

Table 7–Analyte Elution Script Aspirate/Dispense Commands

	Linked Carrier Plate	Liquid Class	Volume (µL)
1	Elution: Elution Buffer	MSIA Workflow	40

Figure 5–MSIA Streptavidin-EVO Analyte Elution Completed Script Reference



3C. Derivatization of MSIA Streptavidin EVO microcolumns with Biotinylated Ligand

The following protocol may be utilized as an initial template for development of a MSIA Streptavidin-EVO workflow. It is recommended that further optimization for each analyte and antibody combination is performed. The procedure may be used to process up to 96 samples in parallel utilizing the MCA 96 head on the Tecan Freedom EVO.

For an example of this workflow being utilized reference the following application note: *A universal LB-MSIA workflow using Freedom Evo platform for the pre-clinical analysis of therapeutic antibodies of differing allotypes in rodent plasma.*

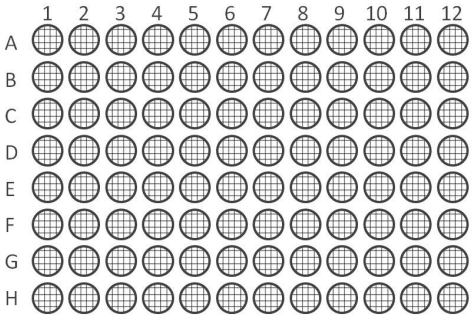
3C.1: Average Time Allotment to Perform the MSIA Streptavidin-EVO Workflow

MSIA Streptavidin-EVO Biotinylated Ligand Capture	
Step	Duration
Preparation of Biotinylated Affinity Ligand Reagents	approx. 15 minutes
Running MSIA EVO Biotinylated Ligand Coupling Script	30 minutes
MSIA Streptavidin-EVO Analyte Capture	
Step	Duration
Preparation of Analyte Capture Reagents and Samples	approx. 20 minutes
Running MSIA EVO Analyte Capture Script	90 minutes
MSIA Streptavidin-EVO Analyte Elution	
Step	Duration
Preparation of Analyte Elution Reagents	approx 10 minutes
Running MSIA EVO Analyte Elution Script	2 minutes

3C.2: Preparation of Reagent Plates

Use the color coded plate maps represented below to add the affinity ligand capture reagents to the corresponding wells of four 96-well microplates. Reference Table 8 for the color key:

Plates 1,3,4 : Rinses; PBS



Plates 2: Biotinylated Affinity Ligand

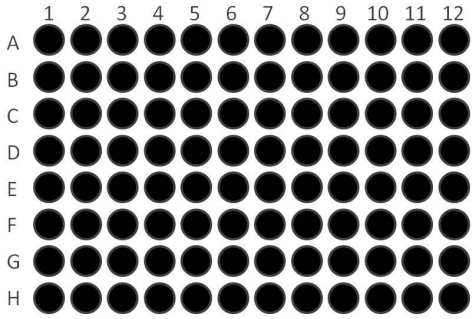


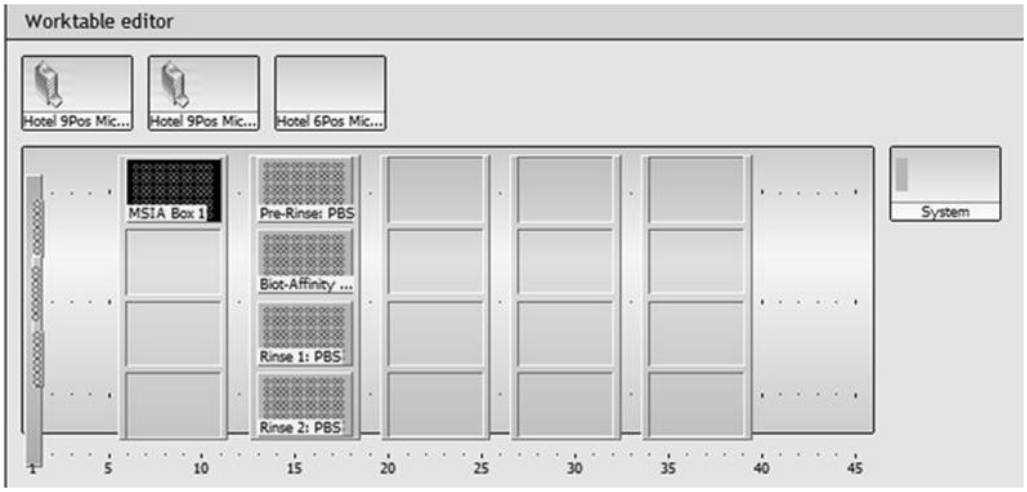
Table 8–Key Code for the 96-Well Plate Maps for the MSIA Streptavidin-EVO Biotinylated Affinity Ligand Capture

Reagent	Representative Texture	Reagent	Plate(s)
Rinse Buffer	Squares	200µL of PBS	1, 3, 4
Biotinylated Affinity Ligand Solution	Solid Black	125 µL of 10µg* of Biotinylated Affinity Ligand in 10mM PBS per MSIA Streptavidin EVO Microcolumn being prepared (up to 96).	2
*10µg of the Affinity Ligand per MSIA Streptavidin EVO microcolumn is a recommended starting point. Optimization of the amount of the affinity ligand will be necessary.			

3C.3: Preparation of the Tecan Freedom EVO Worktable for the Derivatization of MSIA Streptavidin EVO Microcolumns with the Biotinylated Affinity Ligand

1. Open the MSIA EVO Biotinylated Ligand Coupling script file in the Freedom EVOware.
2. Place the prepared reagent plates according to the script file's worktable (Figure 6).

Figure 6–MSIA Streptavidin-EVO Biotinylated Ligand Coupling Worktable



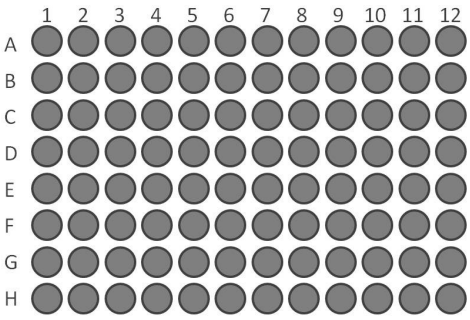
3. Once the worktable is properly setup, close the cover to the Tecan Freedom EVO and begin the script.

3D. Analyte Capture

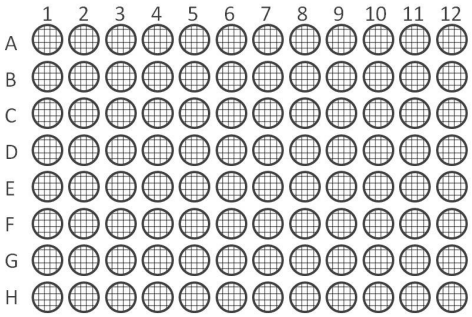
3D.1: Preparation of Reagent Plates

Use the color coded plate maps represented below to add the analyte capture reagents to the corresponding wells of one 96-well deepwell and four 96-well microplates. Reference Table 9 for the color key:

Plate 1: Samples (Deepwell)



Plates 2,3: Rinse; PBS



Plates 4,5: Rinse; Water

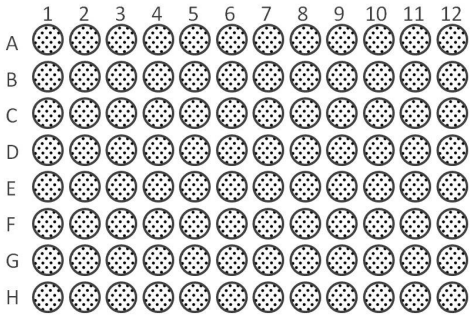


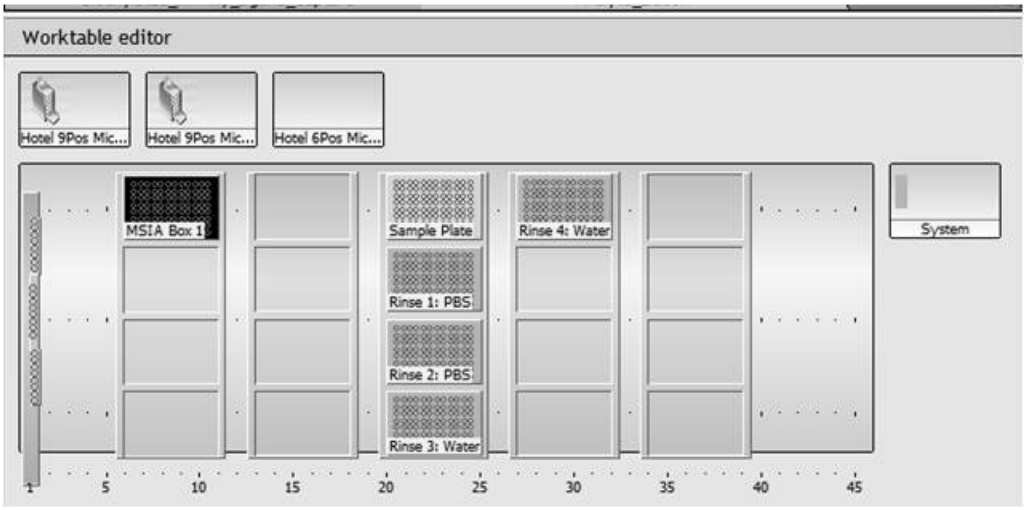
Table 9–Key Code for the 96-Well Plate Maps for the MSIA Streptavidin-EVO Analyte Capture

Reagent	Representative Texture	Reagent	Plate(s)
Rinse Buffer	Squares	200µL of PBS	4, 6, 7
Sample*	Solid Gray	*Refer to Application Note	5 (Deepwell)
Water	Dots	200µL of Optima™ LC/MS Grade Water	8, 9
*An example sample preparation is available in the application note: *A universal LB-MSIA workflow using Freedom Evo platform for the pre-clinical analysis of therapeutic antibodies of differing allotypes in rodent plasma*			

3D.2: Preparation of the Tecan Freedom EVO Worktable for the Analyte Capture

1. Open the MSIA EVO Analyte Capture script file in the Freedom EVOware.
2. Place the prepared reagent plates according to the script file's worktable (Figure 7).

Figure 7—MSIA Streptavidin-EVO Analyte Capture Worktable



3. Once the worktable is properly setup, close the cover to the Tecan Freedom EVO and begin the script.

3E. Analyte Elution

⚠ The MSIA Elution Buffer is a proprietary product recommended for all high throughput applications that reduces loss in signal due to absorption of the protein to the plastics.

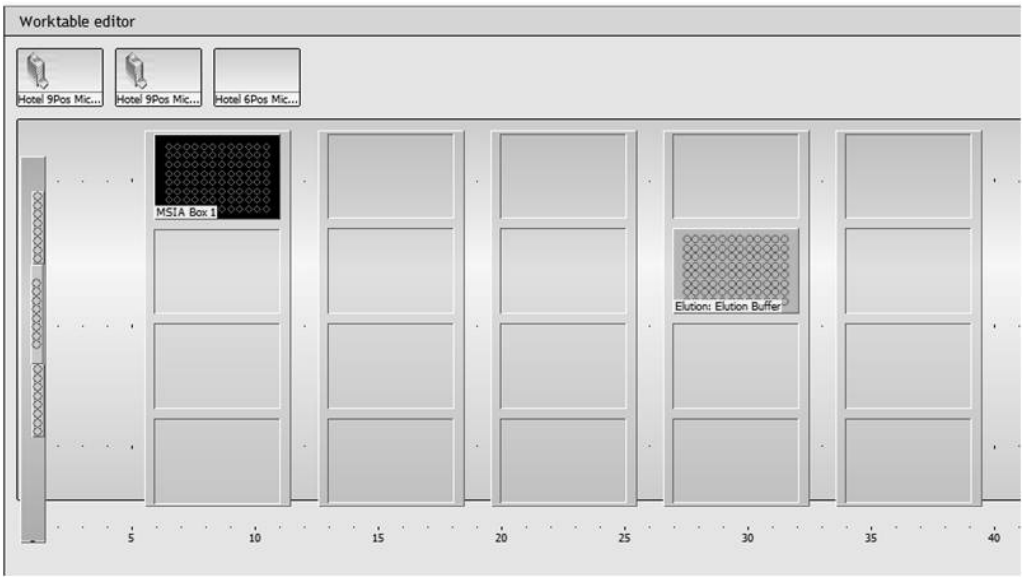
An alternative elution buffer option is to use 0.1% Zwittergent® (Calbiochem™) in 2% Formic Acid with 10% Methanol, however different from the MSIA elution buffer the alternative elution buffer does not prevent loss in signal due to absorption of the protein to the plastics, which worsens with time. If utilizing the alternative elution buffer ensure the samples are loaded onto the LC-MS within one hour of eluting.

For any questions concerning appropriate elution solvents and the MSIA Elution Buffer please use the following contact information:

Technical literature is available at: www.thermoscientific.com/msia
Contact Support: +1 800 345 0206
Outside North America Toll Free: + 1 858 453 7551
Email: msia.info@thermofisher.com

1. The elution will be performed in a 96-Well Polypropylene Plate, 500µL (Elution Plate). Add 100 µL of the MSIA Elution Buffer to all corresponding well (Up to 96).
2. Open the MSIA EVO Analyte Elution script file in the Freedom EVOware.
3. Place the prepared reagent plates according to the script file's worktable (Figure 8).

Figure 8—MSIA Streptavidin-EVO Analyte Elution Worktable



4. Once the worktable is properly setup, close the cover to the Tecan Freedom EVO and begin the script.
5. The eluates are now ready for LC/MS analysis. For an example LC/MS setup please reference the application note: *A universal LB-MSIA workflow using Freedom Evo platform for the pre-clinical analysis of therapeutic antibodies of differing allotypes in rodent plasma*

4. 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 Multichannel Electronic Pipette		
Cat. No.	**Description**	**Packaging**
991CUS02	300µl MSIA D.A.R.T.'S, Custom	Pack of 96 units
991PRT11	300µl MSIA D.A.R.T.'S, Protein A	Pack of 96 units
991PRT12	300µl MSIA D.A.R.T.'S, Protein A	Pack of 24 units
991PRT13	300µl MSIA D.A.R.T.'S, Protein G	Pack of 96 units
991PRT14	300µl MSIA D.A.R.T.'S, Protein G	Pack of 24 units
991PRT15	300µl MSIA D.A.R.T.'S, Protein A/G	Pack of 96 units
991PRT16	300µl MSIA D.A.R.T.'S, Protein A/G	Pack of 24 units
991STR11	300µl MSIA D.A.R.T.'S, Streptavidin	Pack of 96 units
991STR12	300µl MSIA D.A.R.T.'S, Streptavidin	Pack of 24 units
991001096	300µl MSIA D.A.R.T.'S, Insulin	Pack of 96 units
991001024	300µl MSIA D.A.R.T.'S, Insulin	Pack of 24 units
991R	300 µL MSIA D.A.R.T.'S, Reloadable Rack	1 reloadable rack, D.A.R.T.'S are not included
MSIA Streptavidin-EVO for Immunoaffinity Capture		
Compatible with the Tecan™ Freedom EVO® 150 Robotic Platform equipped with a MCA96 liquid handling arm		
Cat. No.	**Description**	**Packaging**
992STR96	500µl MSIA Streptavidin EVO microcolumns	Pack of 96 units
Automated Liquid Handling Platform		
Cat. No.	**Description**	
650-MSIA	MSIA Versette Automated Liquid Handler	
Multichannel Pipettes and Pipette Stand		
Cat. No.	**Description**	**Packaging**
991S	Finnpipette Novus i Adjustable Pipette Stand	1 pipette stand
991SP12	Finnpipette Novus i Electronic 12-Channel Pipette, 30-300µl and Pipette Stand	1 pipette and 1 pipette stand
Liquid Chromatography		
Cat. No.	**Description**	
	Thermo Scientific™ Dionex™ UltiMate® 3000 UHPLC System	
066640	ProSwift™ RP-4H Monolith Column, 1.0 x 250 mm	
Mass Spectrometry and Software		
Description		
Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer		
Thermo Scientific™ Pinpoint Software		
Thermo Scientific™ XCalibur™ Software		
Thermo Scientific™ Protein Deconvolution Software, Version 4.0 with the ReSpect™ algorithm		

thermoscientific.com/msia

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Thermo Fisher Scientific
North America +1 800 995 2787
Outside North America +1 858 453 7551
info.sandiego@thermofisher.com

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