SIGMA-ALDRICH®

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Product Information

Streptavidin IA-MS Immunoaffinity Mass Spectrometry Kit

Catalog Number **MSKT0003** Storage Temperature 2–8 °C

TECHNICAL BULLETIN

THOROUGHLY READ ALL INSTRUCTIONS BEFORE FIRST USE

Product Description

The Streptavidin IA-MS Kit enables a robust, highthroughput assay for quantification of low abundance target proteins in animal and human sera by LC-MS/MS. The plate-based format avoids the potential high cost and LC column blockage associated with bead-based enrichment formats and the automation requirements associated with tip-based enrichment formats.

Figure 1.

Workflow for High-Throughput Streptavidin Plate-based Kit



Sample preparation with the Streptavidin IA-MS Kit can be performed in under 5 hours. The kit consists of a streptavidin coated 96 well plate for immunoaffinity enrichment and reagents for rapid, in-plate trypsin digestion. The resulting digest is ready for injection, with no SPE or other cleanup required.

Components

| Product Description | Catalog Number | Quantity |
|---|-------------------|-------------------------|
| Streptavidin High Binding Capacity Coated Plate | S2577 | 1 plate |
| SOLu-Trypsin | EMS0004 | 4 × 100 μL (1 mg/mL) |
| MS Denaturation Solution | EMS0010 | 10 mL |
| Rapid Trypsin Digestion Buffer | EMS0009 | 30 mL |
| Tris Buffered Saline with TWEEN [®] 20 (TBST) powder, pH 8.0 | T9039 | 1 packet |
| Tris Buffered Saline (TBS) powder, pH 8.0 | T6664 | 1 packet |
| EZ-Pierce™ plate seal | Z721581 | 4 films |

Reagents Required but Not Provided.

- Biotinylated capture molecule, such as:
 - o antibody against analyte
 - o antibody ligand
 - o moderate to strong analyte binder
- Internal standard known to bind with capture molecule, ideally a full-length stable isotope labeled version of the analyte protein
- 88–91% Formic acid (Catalog Number 399388)
- LC-MS grade Water (Catalog Number 1.15333)
- Acetonitrile (Catalog Number 1.00029)

Equipment Required but Not Provided.

- Precision single-channel pipettors certified to deliver 2 μL to 1 mL volumes
- Precision multichannel pipettors certified to deliver 5 μL to 200 μL volumes
- LC column, such as C18 BioShell[™] A160, 0.5 mm × 10 cm × 2.7 μm (Catalog Number 67096-U)
- Orbital shaker
- Thermomixer capable of maintaining 60 °C and 600 rpm
- LC-MS/MS system, such as Sciex 5500

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at 2–8 °C. The kit is stable for two years refrigerated.

Procedure

It is responsibility of the customer to validate the kit for enrichment of their molecule of interest.

Reagent Preparation

- TBST Solution Add contents of one package of Catalog Number T9039 to 1 L of ultrapure water. Shake until fully dissolved.
- TBS Solution Add contents of one package of Catalog Number T6664 to 1 L of ultrapure water. Shake until fully dissolved.
- Capture Molecule Solution Dilute biotinylated capture molecule **(sold separately)** to a final concentration of 10–50 μg/mL in TBST Solution to provide 1–5 μg per well in the conjugation step below.
- Trypsin Working Solution Mix 400 μL of SOLu-Trypsin (Catalog Number EMS0004) with 14.6 mL of Rapid Trypsin Digestion Buffer (Catalog Number EMS0009). SOLu-Trypsin vials may be centrifuged to maximize volume recovery. This solution may be prepared during the immunoaffinity enrichment step.

Preparation of Standards (sold separately)

Prepare a series of calibrators across a 100-fold concentration range in blank matrix or suitable surrogate matrix. Table 1 shows an example of dilution scheme for building a calibration curve through serial 2-fold dilutions.

Table 1.

Example preparation of calibration standards

| Standards | Concentration (ng/mL) |
|-----------|-----------------------|
| Stock | 250 |
| Н | 125 |
| G | 62.5 |
| F | 31.2 |
| E | 15.6 |
| D | 7.81 |
| C | 3.90 |
| В | 1.95 |
| A | 0.98 |

<u>Assay Workflow</u>

Conjugation of Capture Molecule to Plates

- Add 100 μL of Capture Molecule Solution to all wells being used in the analysis. The total amount of capture molecule per well is suggested to be 1–5 μg. The optimal amount is highly dependent on the quality of the antibody and the analytical application. If desired, a capture antibody titration can be performed to determine the optimal amount of capture antibody required to maximize analyte signal.
- 2. Incubate for at least 30 minutes at room temperature while shaking at 150 rpm.
- 3. Empty well contents by inversion and gentle patting on a paper towel.
- Wash wells once with 200 μL of TBST Solution and empty contents by inversion and gentle patting on a paper towel.

Immunoaffinity Enrichment

- To each well, add 200 μL of sample or standard pre-mixed with internal standard. For samples/ standards in the 1–100 ng/mL range, 200 μL of sample spiked with 20 ng/mL internal standard is suggested. For more concentrated samples, premix 5-10 μL of sample with TBS solution and an appropriate amount of internal standard.
- 2. Shake at 150 rpm at room temperature for at least 2 hours.
- 3. Empty well contents by inversion and gentle patting on a paper towel.

- Wash wells once with 200 μL of TBST Solution and empty contents by inversion and gentle patting on a paper towel.
- Wash wells twice with 200 μL of TBS Solution and empty contents by inversion and gentle patting on a paper towel.
 <u>Note</u>: **DO NOT use TBST Solution** in this wash step, as Tween detergent should be washed out prior to digest.

Trypsin digestion

- 1. Add 50 μ L of MS Denaturation Solution to each well and shake at 150 rpm at room temperature for at least 15 minutes.
- Add 150 µL of Trypsin Working Solution to each well (equivalent to 4 µg per well). Seal the plate and incubate at 600 rpm at 60 °C for at least 2 hours. <u>Note</u>: Make sure the plate is tightly sealed to avoid evaporation.
- Remove the seal gently.
 <u>Note</u>: Take care when removing the plate seal to avoid cross-well contamination.
- Quench digest with 5 μL of 88–91% formic acid (Catalog Number 399388). Mix by pipetting samples up and down.
- 5. Reseal the plate and place in autosampler for analysis. Alternatively, digests may be transferred to low protein binding autosampler vials or multiwell plate. Alternative vials/plates should be validated prior to use, as non-specific adsorptive losses can be significant.

LC-MS/MS Analysis

- 1. Inject 10 μL for LC-MS/MS analysis.
- Suggested LC parameters: Column: BioShell C18, A160 0.5 mm × 10 cm × 2.7 μm Column Temperature: 45 °C Auto Sampler Temperature: 8 °C Flow Rate: 25 μL/min LC Mobile Phases: Solvent A: 99.9% H₂O, 0.1% FA Solvent B: 100% ACN Gradient: An appropriate gradient

Legal Information U.S. patents pending

BioShell is a trademark of Sigma-Aldrich Co. LLC. TWEEN is a registered trademark of Croda International PLC. EZ-Pierce is a trademark of Excel Scientific, Inc.

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Appendices Frequently Asked Questions

- Can other diameter analytical columns be used? Columns with larger or smaller diameters than the suggested 0.5 mm diameter may be used for analysis. Column loads and flow rates should be adjusted proportionally to the square of the column diameter.
- 2. Can I process less than 96 samples with this kit? The streptavidin coated plate is comprised of 8 well strips that can be processed separately. Remaining well strips can be removed from the plate frame and stored in a sealed bag with the provided desiccant. Excess reagents should be stored according to Table 3. Allow all reagents to come to room temperature before use.

Table 3.

Storage of Excess Reagents

| Component | Storage |
|--------------------------|--------------------|
| TBST Solution | 2-8 °C for 1 month |
| TBS Solution | 2-8 °C for 1 month |
| Trypsin Working Solution | –20 °C for 1 month |

- 3. What sera have been qualified for use with this kit? The assay is designed to work with animal and human sera. It is recommended the biotinylated molecule be as specific as possible to analyte, with minimal cross-reactivity to other serum proteins.
- Can prepared samples be stored prior to analysis? Sample digests are stable for up to five days at 4 °C when stored in the provided assay plate. Absorptive losses may occur during storage in other vials or plates.

Troubleshooting and Tips

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