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Product Information

Streptavidin IA-MS Immunoaffinity Mass Spectrometry Kit

Catalog Number MSKT0003

Quick Start Workflow

Product Description

This kit enables high-throughput quantification of low abundance target proteins in animal and human sera by LC-MS/MS in less than five hours. Kit components and solution preparations are listed on back of this card. Note: More detailed technical information can be found on the MSKT0003 product page at sigmaaldrich.com/MSKT0003. It is highly recommended to completely read and understand detailed technical information if this is the first time using this kit.

High-Throughput Streptavidin Plate-based Workflow Add biotinylated capture molecule or antibody Incubate 30 min at RT Wash 3X with 200 µL TBST Add sample/standard + internal standard Add up to 200 µL sample/standard Pre-mixed with internal standard (IS) Enrich target analyte and internal standard Incubate 2 hr at RT Wash with 200 µL TBST Wash 2X with 200 µL TBS **Denature proteins** Add 50 µL Denaturation Solution Incubate 15 min at RT **Digest proteins** Add 150 µL Trypsin Working Solution Incubate 2 hr at 60°C, 150rpm **LC-MRM Analysis** Target analyte IS

Components

Product Description	Catalog Number	Quantity
Streptavidin High Binding Capacity Coated Plates	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	<i>Z</i> 721581	4 films

Reagents Required but Not Provided.

- 88–91% Formic acid (Catalog Number 399388)
- LC-MS grade water (Catalog Number 1.15333)
- Acetonitrile (Catalog Number 1.00029)
- Biotinylated capture molecule
- Internal standard known to bind with capture molecule

Equipment Required but Not Provided.

- LC column, such as C18 BioShell™ A160, 0.5 mm × 10 cm × 2.7 µm (Catalog Number 67096-U)
- Precision single-channel pipettors certified to deliver 2 μL to 1 mL volumes
- Precision multichannel pipettors certified to deliver 5 μL to 250 μL volumes
- Orbital shaker, such as Barnstead Thermolyne AROS™ 160 Adjustable Reciprocating Orbital Shaker
- Thermomixer, such as Eppendorf Thermomixer® C
- 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 $^{\circ}\text{C}.$ The kit is stable for two years refrigerated.

Preparation Instructions

Note: Briefly centrifuge all small reagent vials prior to use.

TBST Solution – Add contents of one package (T9039) to 1 L of ultrapure water.

TBS Solution – Add contents of one package (T6664) to 1 L of ultrapure water.

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 μ L of SOLu-Trypsin (EMS0004) with 14.6 mL of Rapid Trypsin Digestion Buffer (EMS0009).

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
С	3.90
В	1.95
A	0.98

LC-MS/MS Analysis

1. Inject 10 μL for LC-MS/MS analysis.

2. Suggested LC parameters: Column: BioShell C18, A160

 $0.5~\text{mm}\times 10~\text{cm}\times 2.7~\mu\text{m}$ 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

U.S. patents pending

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