

Product Information

Protein A/G IA-MS Immunoaffinity Mass Spectrometry Kit

Catalog Number **MSKT0004**

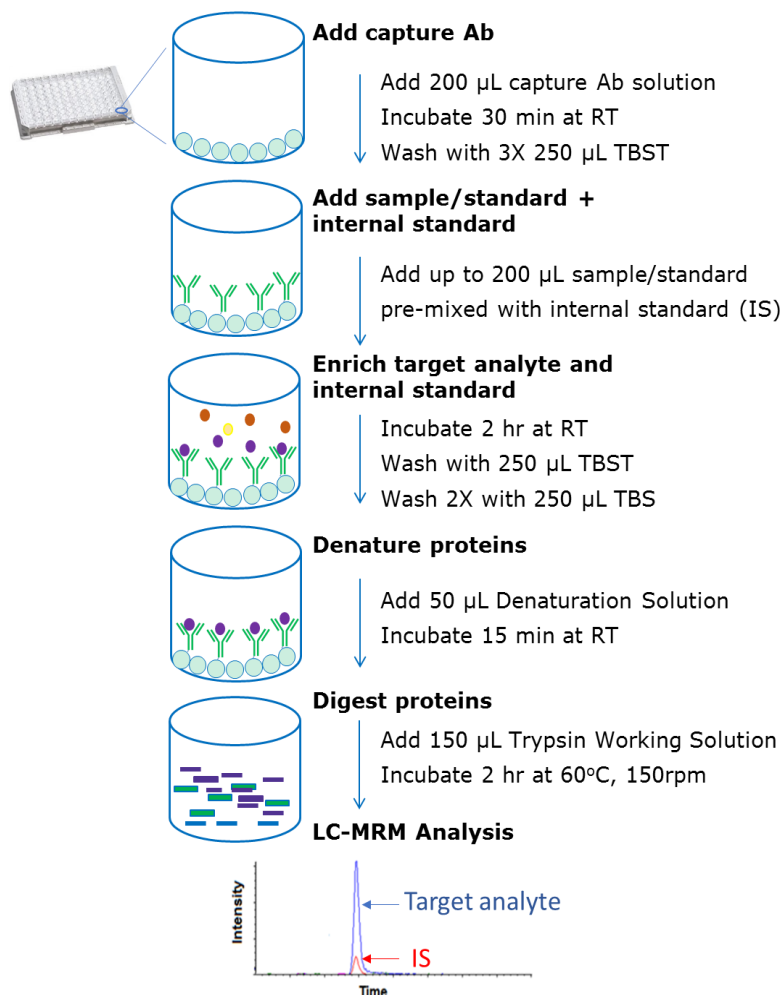
Quick Start Workflow

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 MSKT0004 product page at sigmaaldrich.com/MSKT0004. It is highly recommended to completely read and understand detailed technical information if this is the first time using this kit.

High-Throughput Protein A/G Plate-based Workflow



Components

Product Description	Catalog Number	Quantity
Protein A/G Coated Plates	SAE0113	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.

- 88–91% Formic acid (Catalog Number 399388)
- LC-MS grade water (Catalog Number 1.15333)
- Acetonitrile (Catalog Number 1.00029)
- Capture antibody
- 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 °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 Antibody Solution – Dilute capture antibody (**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 (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
H	125
G	62.5
F	31.2
E	15.6
D	7.81
C	3.90
B	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 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

U.S. patents pending

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