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Product Information

Protein A/G IA-MS Immunoaffinity Mass Spectrometry Kit

Catalog Number **MSKT0004**
Storage Temperature 2–8 °C

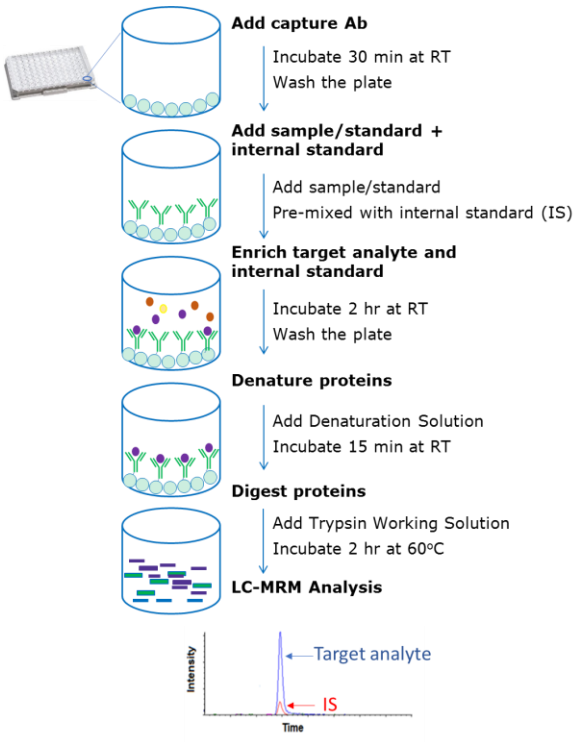
TECHNICAL BULLETIN

THOROUGHLY READ ALL INSTRUCTIONS BEFORE FIRST USE

Product Description

The Protein A/G IA-MS Kit enables a robust, high-throughput 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 Protein A/G Plate-based Kit



Sample preparation with the Protein A/G IA-MS Kit can be performed in under 5 hours. The kit consists of protein A/G coated 96 well plate for immunoaffinity enrichment and 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
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 and Equipment Required but Not Provided.

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

Procedures
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 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 (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
H	125
G	62.5
F	31.2
E	15.6
D	7.81
C	3.90
B	1.95
A	0.98

Assay Workflow

Conjugation of Capture Antibody to Plates

1. Add 100 µL of Capture Antibody Solution to all wells being used in the analysis. The total amount of antibody 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.
4. Wash wells once with 200 µL of TBST Solution and empty contents by inversion and gentle patting on a paper towel.

Immunoaffinity Enrichment

1. 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.

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

Trypsin digestion

1. Add 50 µL of MS Denaturation Solution to each well and shake at 150 rpm at room temperature for at least 15 minutes.
2. 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.
   Note: **Make sure the plate is tightly sealed** to avoid evaporation.
3. Remove the seal gently.
   Note: **Take care when removing the plate seal to avoid cross-well contamination.**
4. 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.
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% H_2O, 0.1% FA
   Solvent B: 100% ACN
   Gradient: An appropriate gradient

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Appendices
Frequently Asked Questions

1. **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 Protein A/G 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 all animal and human sera. It is recommended the capture antibody be as specific as possible to analyte, with minimal cross-reactivity to other serum proteins.

4. **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

Problem	Possible Cause	Solution
The calibration curve is not linear	Pipetting error, poor dilution series	Check pipetting technique and double check calculations.
	Adjacent well cross contamination	Make sure the seal covers the plate completely and use gentle shaking.
The intensity of signature peptide too low	The digestion efficiency is not sufficient	Compare the intensity of peaks with the labeled IS peptide. If both the unlabeled and labeled are not observed or have low intensity, then the problem could be digestion. Make sure the digestion buffer and denaturation buffer have pH >7. Make sure that 50 µL denaturation buffer and 150 µL of Trypsin solution are used. Double check the trypsin concentration.
	Using larger diameter LC column	Larger columns need more volume of sample on the column to obtain the %CV within 20%. Inject >10 µL.
	Check that all reagents have been added in the correct order.	Double check the reagents and procedure and recalculate the sample amount.
	Washes too stringent.	For washing follow the instructions. Make sure not to touch the plate walls while pipetting.
	The capture antibody expired	Check the expiration dates of reagent before use.
	Incorrect assay temperature (too cold)	Follow the instructions for temperature of each step.
	Incorrect assay pH	Check the pH of the sample in TBS buffer before enrichment, make sure the pH is 7–8.5 before use.
	Improper storage of kit	Store all reagents as recommended.
	Non-specific adsorptive losses during storage or analysis.	Sample digests should be analyzed directly in the provided assay plate when possible. If digests must be transferred to alternate vials/plate for analysis, the latter must be validated as low binding to avoid loss in sensitivity.
High variability	Pipetting	Make sure equal amounts of internal standards are used for each well. Dispense the solution into wells quickly and in the same order. Check the pipette calibration and re-run the assay and use appropriate tips.
	Cross well contamination	Make sure the seal is tight while shaking the plate. Hold the plate and gently remove the seal to avoid cross-well contamination.