


# SureQuant™ Streptavidin IP-MS Sample Preparation Kit

Catalog Numbers A51744

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 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

## Product description

The SureQuant™ Streptavidin IP-MS Sample Preparation Kit enables highly effective antigens immunoprecipitation (IP) and co-immunoprecipitation (co-IP) for mass spectrometry (MS) analysis. The high-capacity streptavidin magnetic beads help maximize the recovery of low-abundant targets while their low nonspecific binding minimizes background protein identifications. Cells are first lysed in a non-ionic, detergent-containing buffer and incubated with customer-specific antibody or mixture of antibodies to form an immune complex for each target protein. The bound complexes are thoroughly washed with detergent-free buffers to help reduce nonspecific binding and to remove any residual detergent. A trypsin enzyme elution method is used to dissociate the bound immune complexes from the streptavidin beads, which can then be digested directly in-solution and followed up by MS analysis on a same day; no SDS-PAGE purification is required. The high affinity biotin-streptavidin interaction allows the antigen to be eluted with significantly less antibody contamination compared to other antibody capture ligands such as Protein A, G, or A/G.

## Contents and storage

The SureQuant™ Streptavidin IP-MS Sample Preparation Kit contains reagents sufficient for 20 reactions.

**Table 1** SureQuant™ Streptavidin IP-MS Sample Preparation Kit (Cat. No. A51744)

Components	Amount	Storage
Trypsin Protease	2 × 20 µg	-20°C
Trypsin Storage Solution	250 µL	
Streptavidin Magnetic Beads	1 mL	4°C
IP-MS Cell Lysis Buffer	100 mL	
IP-MS Wash Buffer A	75 mL	
IP-MS Wash Buffer B	40 mL	
IP Elution and MS Sample Prep Buffer	10 mL	
Alkylation Solution	1 mL/vial	
Low Protein-Binding Collection Tubes (1.5 mL)	50 each	
Bond-Breaker™ TCEP Solution, Neutral pH	0.5 mL	
10% Pierce™ Trifluoroacetic Acid (TFA), Sequencing Grade	0.5 mL	

## Additional information

- Do not centrifuge, dry or freeze the magnetic beads, because this can cause the beads to aggregate and lose binding activity.
- Co-elution of antibody with the immunoprecipitated antigen occurs with this kit. MS will identify some heavy and light chain peptides, however, the target identification will not be affected.
- For optimal results, use an affinity-purified antibody. Although serum may be used, the antibody that is specific for the antigen of interest may comprise only 1–2% of the total IgG in the serum sample and will result in low antigen yields.

- IP-MS Cell Lysis Buffer has been tested on representative cell types including, but not limited to: MCF7, HeLa, Jurkat, A431, A549, MOPC, NIH/3T3, HEK293, HCT116, BT-549, and U2OS. Typically, 10<sup>6</sup> HeLa cells yield ~10 mg of cell pellet and ~3 µg/µL (or 300 µg) of protein when lysed with 100 µL of buffer.
- To minimize protein degradation, include protease inhibitors (e.g., Thermo Scientific™ Halt™ Protease and Phosphatase Inhibitor Cocktail (100X), EDTA-free, Product No. 78441) in preparation of cell lysates.
- The IP-MS Cell Lysis Buffer is compatible with the Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Product No. 23225), Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Kit (Product No. 23246) and Thermo Scientific™ Pierce™ 660 nm Protein Assay Kit (Product No. 22662).
- Efficient antibody biotinylation can be accomplished using the Pierce™ Antibody Biotinylation Kit for IP (Product No. 90407).

## Materials required but not provided

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Phosphate-buffered saline (PBS, 100 mM sodium phosphate, 100 mM NaCl; pH 7.2)	<a href="#">28372</a>
Antigen sample (cell lysate or tissue lysate)	<a href="#">MLS</a>
Biotinylated antibody or antibodies	<a href="#">MLS</a>
Vacuum concentrator (e.g., Thermo Scientific™ SpeedVac™ Vacuum Concentrator Kit, or equivalent)	<a href="#">SPD121P</a>
Thermomixer, heat block, or incubator	<a href="#">MLS</a>
Magnetic stand (e.g., Thermo Scientific™ DynaMag™-2 Magnet)	<a href="#">12321D</a>
Pierce™ Water, LC-MS Grade	<a href="#">51140</a>
Low protein-binding microcentrifuge tube, 1.5 mL	<a href="#">90410</a>

## Procedure

### Lyse mammalian cells

Lyse cell monolayer (adherent) cultures

1. Carefully remove culture medium from cells.
2. Wash the cells 3 times with PBS or HBSS.
3. Add ice-cold IP-MS Cell Lysis Buffer (with added protease and phosphatase inhibitor) to the cells and scrape the cells with a squeegee or other scraping device to lyse.

**Table 2 Suggested volume of IP-MS Cell Lysis Buffer to use for different standard culture plates.**

Plate Size/Surface Area	Volume of IP-MS Cell Lysis Buffer
100 × 150 mm	400-800 µL
100 × 100 mm	300-500 µL
100 × 60 mm	100-300 µL
6-well plate	200-400 µL/well
24-well plate	100-200 µL/well

4. Collect cells into a common polypropylene conical tube or equivalent.
5. Incubate on ice for 10 minutes with periodic mixing.

6. Transfer the lysate to microcentrifuge tubes and centrifuge at  $\sim 16,000 \times g$  for 10 minutes to pellet the cell debris.
7. Transfer supernatant to a new tube for protein concentration determination and further analysis.

#### Lyse cell suspension cultures

1. Centrifuge the cell suspension at  $1000 \times g$  for 5 minutes to pellet the cells. Discard the supernatant.
2. Wash cells 3 times by suspending the cell pellet in PBS or HBSS. Centrifuge at  $1,000 \times g$  for 5 minutes to pellet cells.
3. Add ice-cold IP-MS Cell Lysis Buffer (with added protease and phosphatase inhibitor) to the cell pellet. Use 500  $\mu\text{L}$  of IP-MS Cell Lysis Buffer per 50 mg of wet cell pellet (i.e., 10:1 v/w). If using a large amount of cells, first add 10% of the final volume of IP-MS Cell Lysis Buffer to the pellet and pipette the mixture up and down to mix. Add the remaining volume of IP-MS Cell Lysis Buffer to the cell suspension.
4. Incubate lysate on ice for 10 minutes with periodic mixing. Remove cell debris by centrifugation at  $\sim 13,000 \times g$  for 10 minutes.
5. Transfer supernatant to a new tube for protein concentration determination and further analysis.

#### Prepare immune complex

**Note:** The amount of sample needed and the incubation time are dependent upon each specific antibody-antigen system and may require optimization for maximum yield.

1. Combine cell lysate with 5  $\mu\text{g}$  of biotinylated IP antibody per sample in a microcentrifuge tube. The suggested amount of total protein per IP reaction is 500–1,000  $\mu\text{g}$ .
2. Dilute the antibody/lysate solution to 500  $\mu\text{L}$  with IP-MS Cell Lysis Buffer.
3. Ensure tubes are completely sealed and wrap each with paraffin film.
4. Incubate lysate and biotinylated antibody mix at  $4^\circ\text{C}$  overnight with gentle end-over-end rotation to form an immune complex.

#### Manually perform immunoprecipitation and prepare MS samples

**Note:** To ensure streptavidin magnetic bead homogeneity, mix the vial thoroughly by repeated inversion, vortexing, or using a rotating platform.

1. Place 25  $\mu\text{L}$  (0.25 mg) of Pierce™ Streptavidin Magnetic Beads into a 1.5 mL low protein-binding microcentrifuge tube (Product No. [90410](#)).
2. Add 175  $\mu\text{L}$  of IP-MS Cell Lysis Buffer to the beads and gently vortex to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step once.
3. Quick spin the antigen sample/antibody mixture (from “Prepare immune complex” on page 3) by centrifugation and add to the tube containing pre-washed magnetic beads. Incubate at room temperature for 1 hour with end-over-end rotation.
4. Quick spin by centrifugation. Collect the beads with a magnetic stand and completely remove supernatant so that no liquid remains.
5. Add 500  $\mu\text{L}$  of IP-MS Wash Buffer A to the tube and mix by vortexing for 10–15 seconds. Collect the beads and carefully remove the supernatant. Perform this wash a total of 3 times.
6. Add 500  $\mu\text{L}$  of IP-MS Wash Buffer B to the tube and mix by vortexing for 10–15 seconds. Collect the beads and carefully remove the supernatant. Perform this wash a total of 2 times. Prior to last wash, quick spin by centrifugation to avoid bead loss.
7. Prepare 0.2  $\mu\text{g}/\mu\text{L}$  trypsin by adding 100  $\mu\text{L}$  of trypsin storage solution to 20  $\mu\text{g}$  trypsin in a vial. Pipette solution 5–10 times to solubilize the trypsin and keep the enzyme solution on ice until use.
8. Per each sample, prepare trypsin elution by adding 5  $\mu\text{L}$  of 0.2  $\mu\text{g}/\mu\text{L}$  trypsin to 95  $\mu\text{L}$  of IP Elution and MS Sample Prep Buffer and add to beads. Incubate at  $37^\circ\text{C}$  for 1 hour using a thermomixer set to 1,400 rpm. For optimal results, the mixing speed is critical.
9. Quick spin by centrifugation and collect the beads by placing on a magnet. Transfer 90  $\mu\text{L}$  of the supernatant containing the target antigens to a new 1.5 mL low protein-binding collection tube.
10. Prepare reducing solution by diluting TCEP 1:3.33 (100  $\mu\text{L}$  = 30  $\mu\text{L}$  TCEP + 70  $\mu\text{L}$  Pierce™ Water, LC-MS Grade).

11. Add 10  $\mu\text{L}$  of reducing solution followed by 50  $\mu\text{L}$  of alkylation solution to each sample and incubate at 95°C for 5 minutes.
12. After reduction/alkylation, place samples at 4°C for 1 minute.
13. Add 7  $\mu\text{L}$  of IP Elution and MS Sample Prep Buffer and 3  $\mu\text{L}$  of 0.2  $\mu\text{g}/\mu\text{L}$  trypsin to each sample. Store remaining trypsin at -20°C.
14. Vortex briefly and incubate for 2 hours at 37°C using a thermomixer set to 500 rpm.
15. After trypsin digestion, remove the samples, add 10  $\mu\text{L}$  of 10% TFA, then vortex briefly to stop the digestion. Before proceeding, we recommend confirming that the pH is below 3.0.
16. Centrifuge sample for 2 minutes at 15,000  $\times g$  to remove any remaining particulates.
17. Remove 135  $\mu\text{L}$  of supernatant and transfer to a new low protein-binding collection tube.
18. Dry the supernatant in a speed vacuum concentrator.
19. Once reconstituted, clean-up with C18 spin tips (Product No. 87784) or C18 trap column (online/offline) before MS analysis.  
**Note:** For LC-MS/MS analysis, use of online C18 trap column is recommended to clean up sample to avoid sample loss.

## Troubleshooting

Observation	Possible cause	Recommended action
Antigen(s) did not immunoprecipitate or low amount of recovered protein.	Sample did not contain sufficient antigen to detect.	Verify protein expression and/or lysis efficiency by SDS-PAGE or Western blot. Use more lysate for IP if required.
	Protein degraded.	Add protease and phosphatase inhibitors.
	Insufficient magnetic beads used.	Ensure magnetic beads are evenly suspended before use.
	Short liquid-chromatography (LC) gradient and slow-scanning mass spectrometer were used.	Use nano LC with longer gradient (60 minutes) and fast-scanning mass spectrometer (e.g., Thermo Scientific™ Q Exactive™/Orbitrap™ Fusion Mass Spectrometer).
	Low recovery of peptides after in-solution digestion.	Use low protein-binding microcentrifuge tubes (provided in kit) for maximum recovery of target peptides .
High non-specific background proteins identified.	Non-specific proteins bound to the magnetic beads.	Pre-clear sample by incubating with Pierce™ Streptavidin Magnetic Beads without antibody before forming the immune complex.
Magnetic beads aggregated.	Magnetic beads were frozen or centrifuged.	Handle the beads as directed in the instructions.

## Limited product warranty

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](http://thermofisher.com/symbols-definition).

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Revision	Date	Description
C.0	21 June 2023	Inserted new step 14 in Manually perform immunoprecipitation and prepare MS samples. Added single product LULL statement.
B.0	3 August 2021	Updated the product name.
A.0	12 July 2021	New document for SureQuant™ Streptavidin IP-MS Sample Preparation Kit.

The information in this guide is subject to change without notice.

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