



MICROSCOOP[®] / MINT

Spatial Proteomics with MICROSCOOP[®] Instructions for use of Synpull[™] Kit 2.0

The reagent for low-volume pulldown and mass spectrometry-ready preparation.

Catalog Number

SYN-PU0206 (Up to 6 reactions)

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FOR RESEARCH USE ONLY.

LB03.B.0005

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1. INTENDED USE

The Synpull™ Kit 2.0 is designed to enrich and purify biotinylated proteins from photolabeled cell or tissues after Synlight-Rich™ Kit treatment. Following sample lysis, protein extraction, affinity purification, on-bead digestion and peptide desalting steps, proteins from regions of interest (ROI) can be detected by LC-MS/MS analysis. All reagents in Synpull™ Kit are for research use only (RUO).

2. INTRODUCTION

The Synpull™ Kit 2.0 provides instructions and materials needed to extract biotinylated proteins from regions of interests (ROIs) that have been photolabeled using the Synlight-Rich™ Kit and Microscope® Mint (CATALOG NUMBER: SYN-RI0106, SYN-RI0206). For high-confidence identification of protein candidates, it is advised to process paired samples (both unlabeled and photolabeled samples) simultaneously and analyze them within the same LC-MS/MS batch. Each Synpull™ Kit provides sufficient material for performing six reactions, which is equivalent to three pairs of samples.

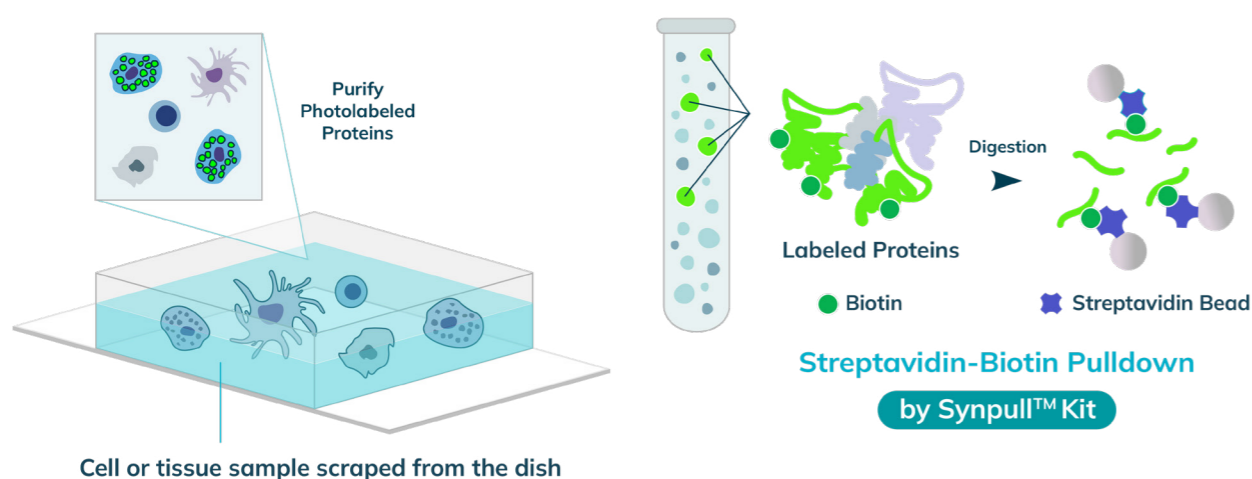


Fig. 2 Principle of Synpull™ Kit

3. KIT CONTENTS AND REAGENTS

3.1 Catalog Number SYN-PU0206 (Up to 6 reactions)

Cap/Bag Label	Component	Quantity	Dilution Factor	Ref. No.
A	Scrape	4.32 mL	1×	SYN-PU02RA
B	Lyse 1	3.3 mg (powder)	N/A	SYN-PU02RB
C	Lyse 2	360 µL	1×	SYN-PU02RC
D	Lyse 3	15.2 mg (powder)	N/A	SYN-PU02RD
E	Dilute	4 mL	1×	SYN-PU02RE
F	Pull	80 µL	N/A	SYN-PU02RF
G	Wash 1	6 mL	1×	SYN-PU02RG
H	Wash 2	6 mL	1×	SYN-PU02RH

I	Wash 3	6 mL	1×	SYN-PU02RI
J	Wash 4	7.35 mL	1×	SYN-PU02RJ
K	Digest	1.2 µg (powder)	N/A	SYN-PU02RK
L	Stop	12 µL	1×	SYN-PU02RL
M	Desalt 1	1.2 mL	1×	SYN-PU02RM
N	Desalt 2	1.2 mL	1×	SYN-PU02RN
O	Desalt 3	120 µL	1×	SYN-PU02RO
P	Verify	120 µL	50×	SYN-PU02RP
CA	Proteomics tube	18 tubes	N/A	SYN-PU02CA
CB	Desalting tip	6 tips	N/A	SYN-PU02CB

3.2 Warnings and Precautions

- RUO only.
- Do not use kits beyond the expiration date.
- If the reagent solution comes in contact with the skin or eye, flush with copious amounts of water.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.

4. MATERIALS REQUIRED BUT NOT PROVIDED

4.1 Consumables

Item	Description	Supplier	Cat. No.
Cell scraper	For collecting cell or tissue samples	TPP	99002
1.7 mL tubes	Regular tubes as primary container for materials	Axygen	MCT-175-C
Low-retention tips	For minimizing sample loss for 1 mL, 0.2 mL, 20 µL, 10 µL pipettors	SMB	T series
Ultrapure water	For sonicator probe cleansing; freshly prepared ultrapure water		
Methanol	For sonicator probe cleansing; laboratory grade	Sigma	34860

Protein Assay Kits & IDCR	Essential for determining extracted protein concentration. Recommend using Pierce 660nm protein assay with IDCR; BCA or DC assay results will be underestimated.	Pierce	22662; 22663
96-well plates	For performing protein assays (Nunc)	Thermo Fisher Scientific	15041

4.2 Equipment

Item	Description
Laboratory Grade 4°C Refrigerator/ -20°C freezer	Reagent storage
Single Channel Pipettes	For sample handling and pipetting, the dispensing volume range is 2~1000 µL
Centrifuge (5000 or 16000 × g)	For spinning down cell/tissue samples
Vortex	For mixing or washing samples at 1000 rpm
Minicentrifuge	For spinning down reagents or samples in 1.5 mL tubes
Sonicator/ Disruptor	For performing cell (ultrasonic water bath)/ tissue (sonicator probe or disruptor) lysis
Heat block	For performing cell/tissue lysis at temperature between 80-100°C
Plate/ELISA reader	For measuring the absorbance of standards and unknown samples from the protein extraction at 660 nm
ELMI Intelli-Mixer/Syncell provided with instrument	Highly recommended for rotating or shaking samples at a small angle (CATALOG NUMBER: RM-2L/M/S, ELMI)
Magnetic rack	For working with magnetic beads separation (CATALOG NUMBER: 12321D, Invitrogen)
Ultrasonic bath	For cell lysis and resuspension of magnetic beads
Oven/incubator (37°C)	For on-bead digestion at 37°C; the ELMI Intelli-Mixer is placed inside the oven/incubator
SpeedVac concentrator	For evaporating buffers from samples
Biological safety cabinet (BSC) or laminar flow hood	For keratin-free environment

5. STORAGE

- **Digest (K)** should be stored at -20°C (-15 to -30°C) upon receipt.
- All other components of the kit should be stored at 4°C (2 to 8°C).
- **Pull (F)** should not be stored below 0°C.

When stored under these conditions, the kit components will remain stable until the expiration date indicated on the outer packaging. Immediately after reagent preparation, store any unused **Lyse 1 Solution (B)**, **Lyse 3 Solution (D)**, and **Digest Solution (K)** at -20°C before expiration date.

Please ensure that Lyse 1 Solution (B), Lyse 3 Solution (D), and Digest Solution (K) are not subjected to more than three

freeze-thaw cycles.

6. SAMPLE PREPARATION

6.1 Compatible Sample types

To ensure accurate and reproducible results, select an appropriate sample type for use with the Synpull™ Kit. The Synpull kit was validated only with the samples listed below:

- Fixed cells
- Formalin-fixed paraffin-embedded (FFPE) tissue sections
- Frozen (OCT-embedded) tissues

Prepare samples according to the Synlight-Rich™ Kit guidelines. Samples must be immersed in a photoactivatable probe for photolabeling of regions of interest (ROIs), which is conducted using Microscoop®.

6.2 Positive Control (PC)

To verify procedural accuracy, nuclei-labeled cells can be utilized as a positive control (PC) sample to ensure the reliability of experimental procedures.

- Preparation: Follow the appendix of the Synlight-Rich™ Kit manual to prepare nuclei-labeled cells.
- Recommended PC Sample Sets: Prepare a pair of PC samples, consisting of one unlabeled (UL) slide and one photolabeled (PL) slide.

6.3 Reference for Sample Quantity

For optimal results, the table below provides reference to the required sample quantity:

Category	Cell line/ Tissue type	Target and size (top view)	No. of slides	No. of cells/ area size per slide
Cell	U-2 OS	Nucleus (~20 µm ²)	2 (UL); 2 (PL)	2.4 x 10 ⁵
	U-2 OS	Mitochondrion (~0.8-3.0 µm ²)	3 (UL); 3 (PL)	2.4 x 10 ⁵
	RPE-1	Cilium (~0.2-0.7 µm ²)	6 (UL); 6 (PL)	2.0 x 10 ⁵
Tissue	Mouse brain	Nucleus (~20 µm ²)	3 (UL); 3 (PL)	300 mm ²

7. TEST PROCEDURES

These procedures outline the detailed steps for sample preparation, including lysis, affinity purification, on-bead digestion, and peptide desalting. Proper adherence to these steps is crucial for obtaining accurate and reliable results. Additionally, it is strongly recommended to frequently refer to the notes provided to ensure the correct preparation and handling of samples and reagents.

Icons: Throughout this guide, you'll see a few helpful icons. Please refer to the descriptions below.



Special note



Signifies critical step

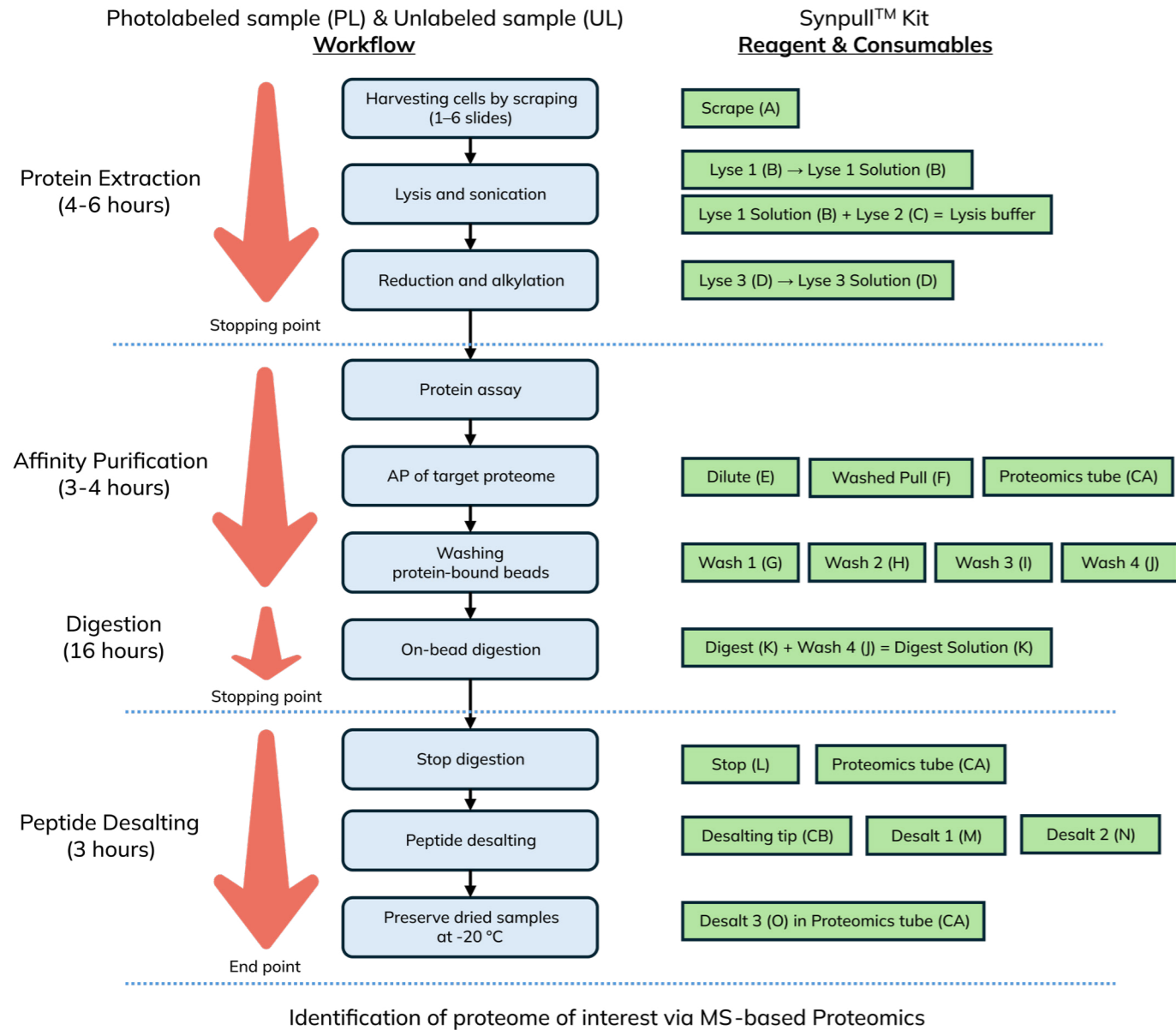


Stopping point

Precautions:

- Liquid reagents - vortex and spin down all reagent before use.
- Solid reagents - equilibrate to RT (room temperature, 15 to 25°C), spin down before opening or dissolving.
- Use low retention tips for all procedures to minimize sample loss.
- Change pipette tips to prevent sample contamination, especially between unlabeled and photolabeled samples.
- All procedures can be performed at RT in a biological safety cabinet (BSC) or laminar flow hood.

7.1 Summary of Synpull™ Kit Workflow



Synpull Kit operation video is available at the QR code below:



7.2 Protein Extraction

7.2.1 Reagent Set-Up and Preparation

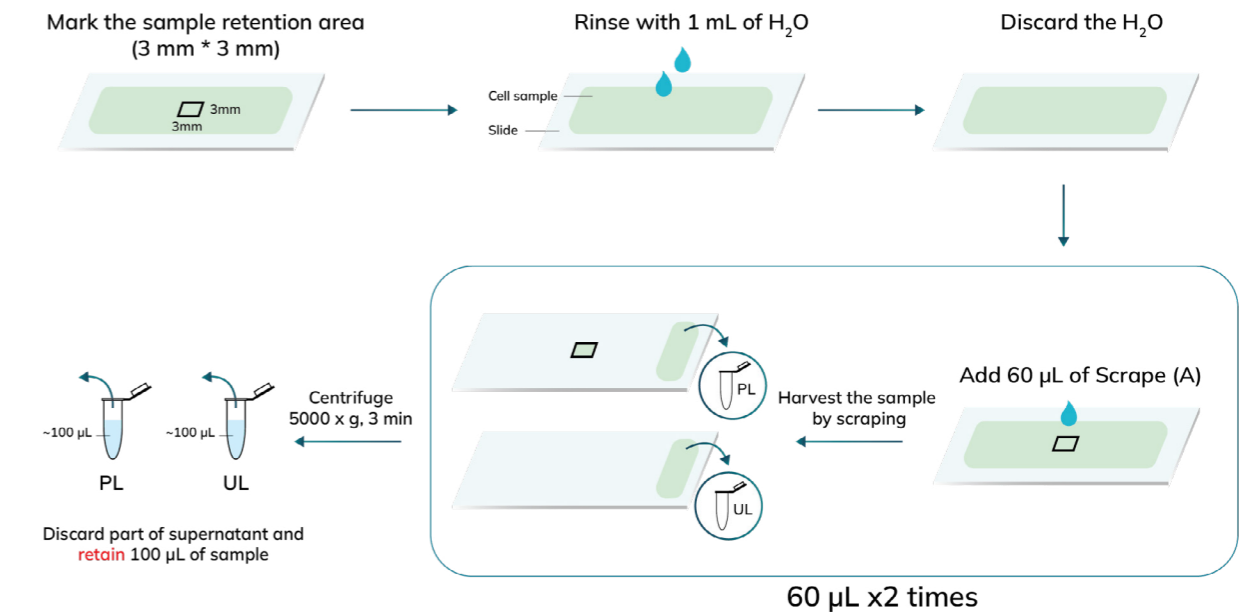
Solution	Handling and Preparation	Storage
Scrape (A)	Mix it gently and ensure buffer is transparent.	4°C
Lyse 1 Solution (B)	Spin down before opening, dissolve Lyse 1 (B) in 50 µL of ultrapure water.	-20°C
Lyse 2 (C)	Thaw at room temperature. Vortex at 1000 rpm until it appears as a clear solution.	4°C
Lyse 3 Solution (D)	Spin down before opening, dissolve Lyse 3 (D) in 100 µL of ultrapure water.	-20°C

For cell samples extraction, please follow sections 7.2.2 and 7.2.3.

For tissue samples extraction, please follow sections 7.2.4 and 7.2.5.

7.2.2 Cell Sample Harvesting

Overview



Step 1: Discard the buffer in the sample well of the slide.

Step 2: Mark a 3 mm² area at the bottom of the slide as the sample retention area. This area will be used to verify the photolabeling efficiency using Verify (P), following the Synlight-Rich™ Kit instructions.



Note 1: If your sample volume is extremely limited, you may skip this step. However, it is highly recommended to perform verification.

Step 3: Rinse both unlabeled and photolabeled samples with 1 mL of ultrapure water, then gently discard the ultrapure water.

Step 4: Prepare the sufficient amount of Scrape (A) for the planned number of slides as per table below and scraping unlabeled (UL) and photolabeled (PL) samples separately. To prevent contamination, distribute the buffers into separate tubes, one for UL and one for PL.

No. slides	1	2	3	4	5	6
Scrape (A), μL	120	240	360	480	600	720

Step 5: Add 60 μL of Scrape (A) into each single-well slide. Harvest the cell samples by scraping, then transfer the sample–buffer mixture to a clean 1.5 mL tube labeled “UL” or “PL.”

Step 6: Repeat Step 5 to ensure that all samples are thoroughly harvested into their respective tubes (UL or PL). Each kit’s Scrape (A) is sufficient for processing up to six slides..

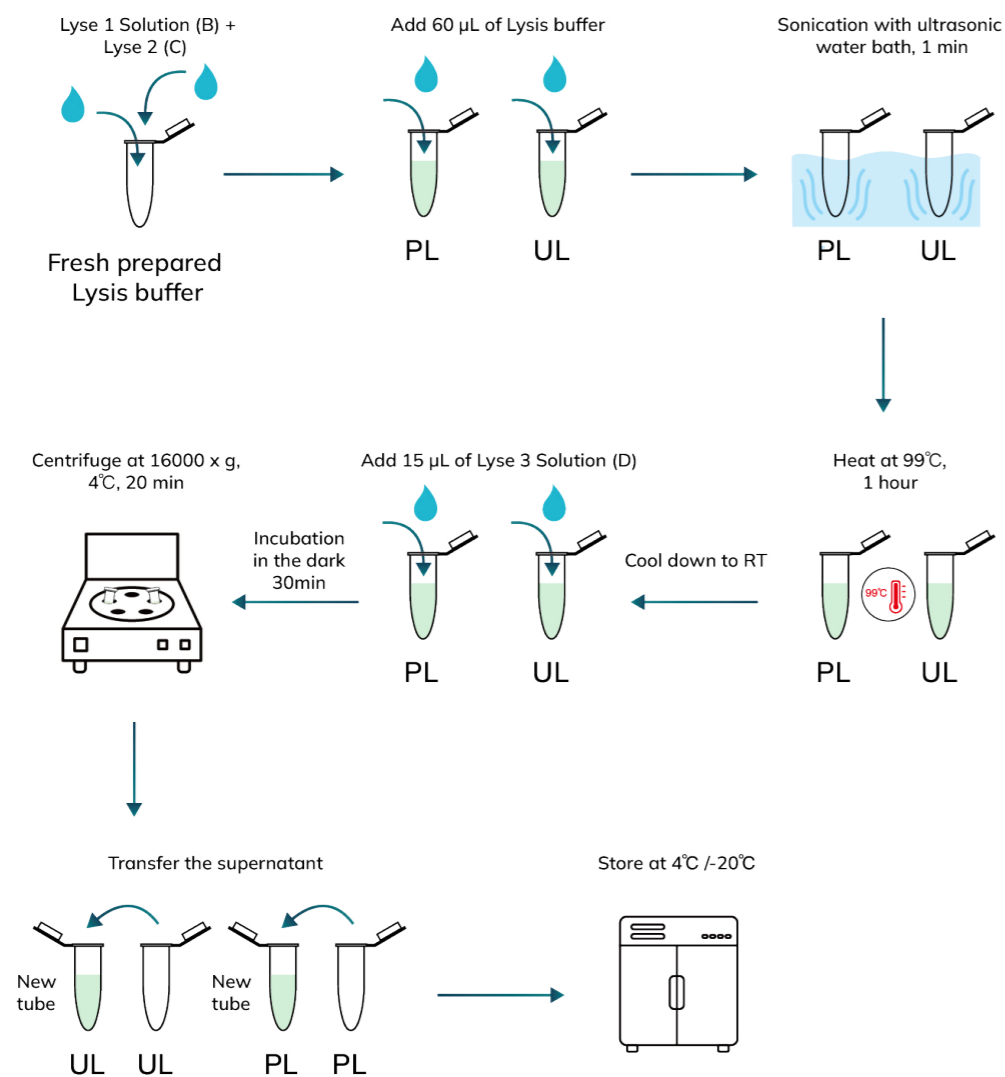
Step 7: Centrifuge the harvested samples at $5000 \times g$ for 3 minutes at 4°C . Carefully discard the supernatant and retain approximately 100 μL of the sample–buffer mixture.



Note 2: As an alternative, a SpeedVac concentrator can be used to evaporate the sample–buffer mixture down to approximately 100 μL . Do not allow the sample to dry completely. If needed, adjust the volume after evaporation with ultrapure water.

7.2.3 Cell Sample Lysis

Overview



Step 1: Pre-heat the heating block to 99°C .

Step 2: Spin down Lyse 1 Solution (B) and Lyse 2 (C).

Step 3: Freshly prepare Lysis Buffer by combining Lyse 1 Solution (B) and Lyse 2 (C) into a tube according to the number of samples. Vortex and spin down.



Note 3: Return Lyse 1 Solution (B) to -20°C after use.

No. samples	2	3	4	5	6
Lyse 1 Solution (B), μL	15	23	30	38	45
Lyse 2 (C), μL	117	176	234	293	351

Step 4: For each sample–buffer mixture (approximately 100 μL), add 60 μL of Lysis Buffer, ensuring a total volume of approximately 160 μL .

Step 5: Lyse the cells by placing the sample–buffer mixture for 1 min in an ultrasonic water bath.



Note 4: To avoid splashing and evaporation, always use an ultrasonic water bath for cell samples.
Note 5: To enhance protein yield, extend the sonication (or disruptor) time until a trace amount of recognizable pellet is visible.

Step 6: Spin down the sonicated samples, then heat them at 99°C for 1 hr.

Step 7: Spin down and allow the heated samples to cool down to RT.

Step 8: Spin down and add 15 μL of Lyse 3 Solution (D) to the cooled samples, then vortex and spin down. Incubate the mixture at RT for 30 minutes in the dark.



Note 6: Return Lyse 3 Solution (D) to -20°C after use.

Step 9: Centrifuge the samples at $16000 \times g$ for 20 min at 4°C .

Step 10: Ensure that the supernatant is transparent. Carefully transfer the supernatants (lysates) into new 1.5 mL tubes (labeled UL or PL tubes) and record the volume for total protein quantification.

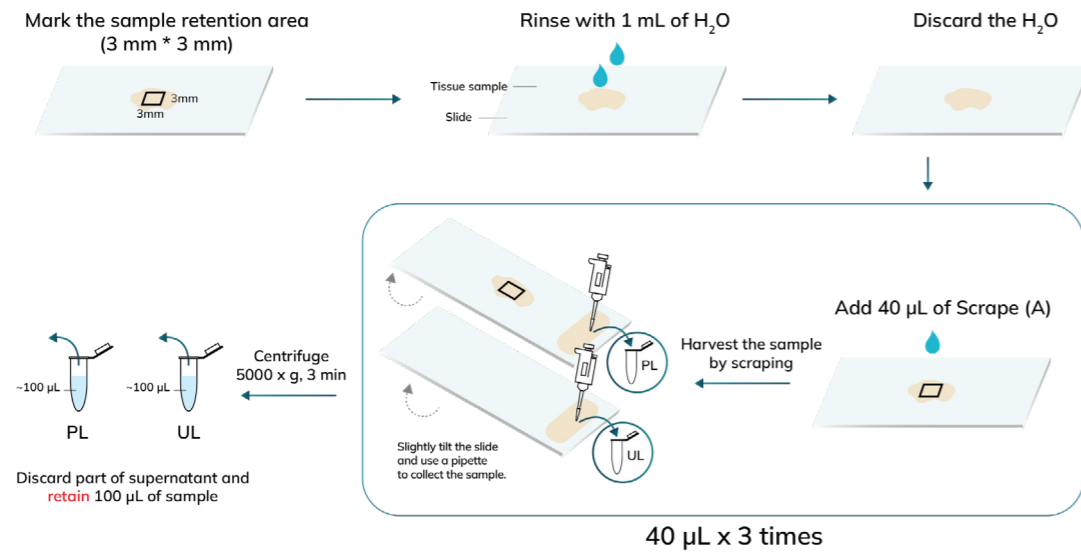
Stopping Point

After this step, store the lysates at 4°C and perform affinity purification within 24 hours. Alternatively, store the lysates at -20°C for up to 1 month before performing affinity purification.

Continue to Section 7.3: Affinity Purification and On-Bead Digestion.

7.2.4 Tissue Sample Harvesting

Overview



Step 1: Discard the buffer in the sample of the glass slide.

Step 2: Mark a 3 mm² area at the bottom of the slide as the sample retention area. This area will be used to verify the photolabeling efficiency using Verify (P), following the Synlight-Rich™ Kit instructions.

Step 3: Rinse both unlabeled and photolabeled samples with 1 mL of ultrapure water, then gently discard the ultrapure water.

Step 4: Prepare the sufficient amount of Scrape (A) for the planned number of slides as per table below and scraping unlabeled (UL) and photolabeled (PL) samples separately. To prevent contamination, distribute the buffers into separate tubes, one for UL and one for PL.

No. slides	1	2	3	4	5	6
Scrape (A), µL	120	240	360	480	600	720



Note 7: If your sample volume is extremely limited, you may skip this step. However, it is highly recommended to perform verification.

Step 5: Add 40 µL of Scrape (A) to each glass slide. Harvest the tissue samples by scraping, then transfer the sample–buffer mixture to a clean 1.5 mL tube labeled “UL” or “PL.”

Step 6: Repeat Step 5 twice to ensure that all samples are thoroughly harvested into their respective tubes (UL or PL). Each kit’s Scrape (A) is sufficient for processing up to six slides.

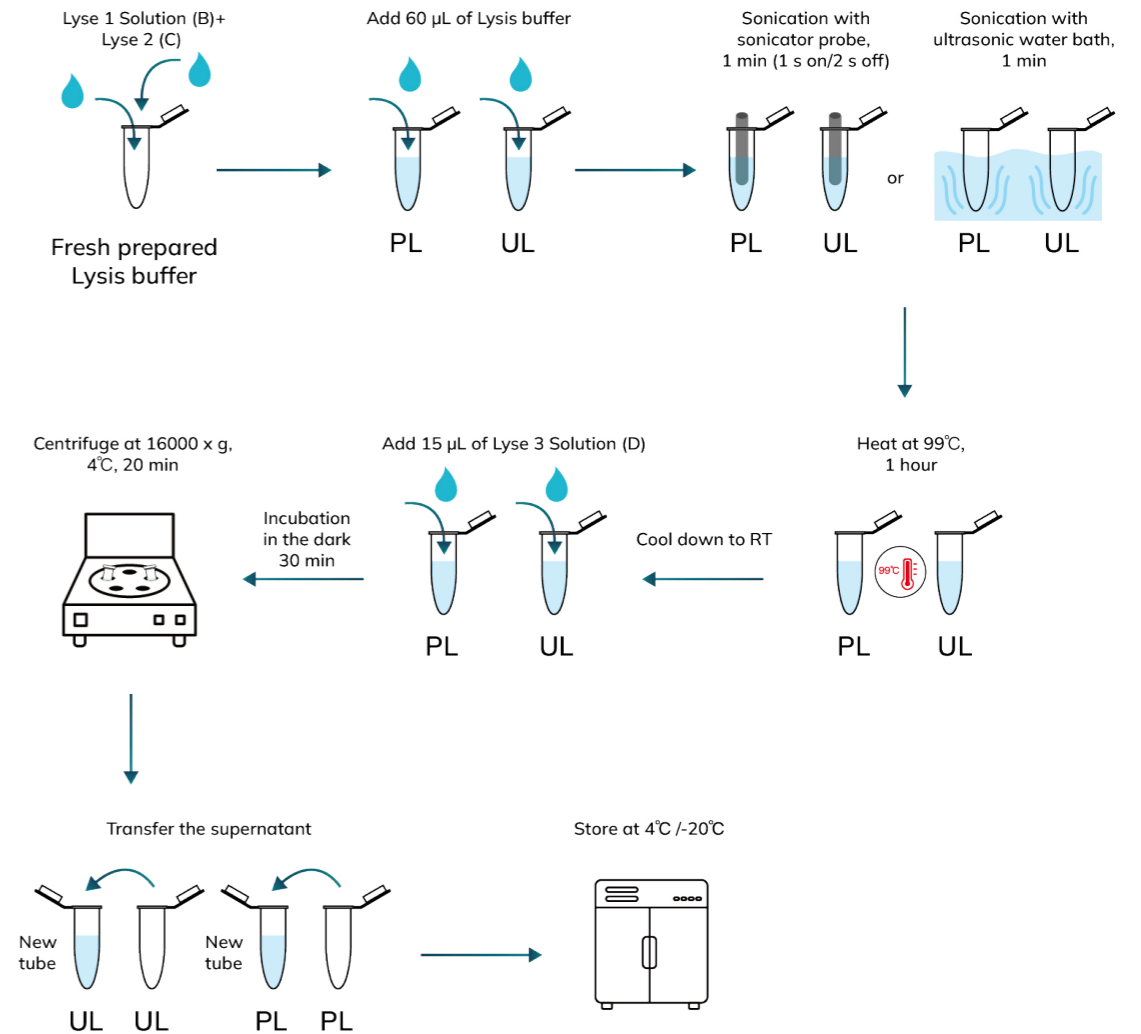
Step 7: Centrifuge the harvested samples at 5000 × g for 3 minutes at 4°C. Carefully discard the supernatant and retain approximately 100 µL of the sample–buffer mixture.



Note 8: As an alternative, a SpeedVac concentrator can be used to evaporate the sample–buffer mixture down to approximately 100 µL. Do not allow the sample to dry completely. If needed, adjust the volume after evaporation with ultrapure water.

7.2.5 Tissue Sample Lysis

Overview



Step 1: Pre-heat the heating block to 99°C.

Step 2: Spin down Lyse 1 Solution (B) and Lyse 2 (C).

Step 3: Freshly prepare Lysis Buffer by combining Lyse 1 Solution (B) and Lyse 2 (C) according to the number of samples into a tube. Vortex and spin down.



Note 9: Return Lyse 1 Solution (B) to -20°C after use.

No. samples	2	3	4	5	6
Lyse 1 <u>Solution (B)</u> , µL	15	23	30	38	45
Lyse 2 (C), µL	117	176	234	293	351

Step 4: For each sample–buffer mixture (approximately 100 µL), add 60 µL of Lysis Buffer, ensuring a total volume of approximately 160 µL.

For tissue samples, it is recommended to use the sonicator probe or disruptor to homogenize the samples described in Steps 6-10.



Note 10: To avoid splashing and evaporation, we recommend using 30% amplitude for lysis with 160 µL of sample, and 60% amplitude for washing probe with 1 mL of ultrapure water/methanol. You may adjust the settings based on your equipment, as long as splashing and evaporation are avoided.



Note 11: To improve the protein yield, extend the sonication (or disruptor) time to achieve a trace amount of recognizable pellet.

Step 5: If the sonicator probe will be used for lysing, prepare N + 1 of 1-mL ultrapure water and N + 1 of 1-mL methanol in 1.5-mL tubes to clean the probe between samples. Typically, N equals 2 (the UL and PL samples).

Step 6: Clean the sonicator probes as follows:

Step 6.1: Submerge the probe in 1 mL ultrapure water. Sonicate for 10 cycles of 1 s on/2 s off at 60% amplitude.

Step 6.2: Submerge the probe in 1 mL methanol. Sonicate for 10 cycles of 1 s on/2 s off at 60% amplitude.

Step 7: Sonicate the sample–buffer mixture for 60 cycles of 1 s on/2 s off at 30% amplitude.

Step 8: Follow Step 6 to wash the sonicator probe using fresh ultrapure water and methanol.

Step 9: Repeat Steps 7–8 to sonicate all sample–buffer mixtures.

Step 10: Spin down the sonicated samples, then heat them at 99°C for 1 hr.

Step 11: Spin down and allow the heated samples to cool down to RT.

Step 12: Spin down and add 15 µL of Lyse 3 Solution (D) to the cooled samples, then vortex and spin down. Incubate the mixture at RT for 30 minutes in the dark.



Note 12: Return Lyse 3 Solution (D) to -20°C after use.

Step 13: Centrifuge the samples at 16000 × g for 20 min at 4°C.

Step 14: Ensure that the supernatant is transparent. Carefully transfer the supernatants (lysates) into new 1.5 mL tubes (labeled UL or PL tubes) and record the volume for total protein quantification.

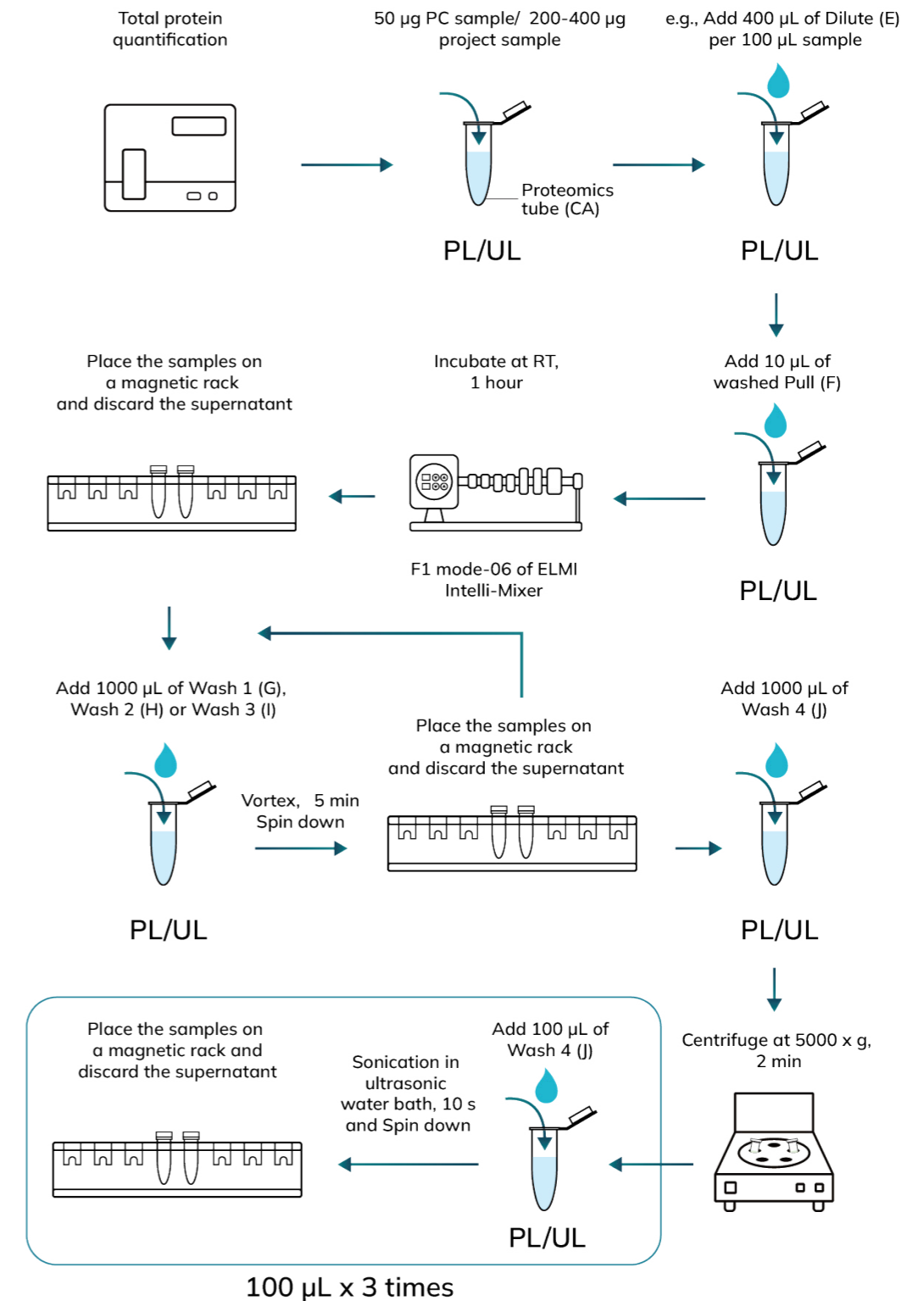
Stopping Point

After this step, store the lysates at 4°C and perform affinity purification (AP) within 24 hours. Alternatively, store the lysates at -20°C for up to 1 month before performing affinity purification.

Continue to Section 7.3: Affinity Purification and On-Bead Digestion.

7.3 Affinity Purification and On-Bead Digestion

Overview of affinity purification



7.3.1 Total Protein Quantification

Determine the protein concentrations in the lysates using the Pierce 660nm Protein Assay (IDCR method):

Step 1: Add 10 μL of protein standards (2.000 – 0.125 mg/mL BSA, provided in the 660nm Protein Assay kit) and diluted lysates (e.g., dilution factor from 1 to 6 with ultrapure water dependent on pulled number of slides) to a 96-well plate. An example is shown below:

Well no.	1	2	3	4	5	6	7	8
Contents	2.000 mg/mL BSA	1.000 mg/mL BSA	0.500 mg/mL BSA	0.250 mg/mL BSA	0.125 mg/mL BSA	Lysate (UL)	Lysate (PL)	Blank (optional)

Step 2: Weigh an appropriate amount of IDCR in a 1.5 mL tube bases on number of slides.

- Example: Weigh 60 mg of IDCR for one measurement of 5 standards, 2 lysates and 1 extra (8 tests).

Step 3: Dissolve IDCR (Ionic Detergent Compatibility Reagent, CATALOG NUMBER: 22663, Pierce) in the 660 nm Protein Assay Reagent (CATALOG NUMBER: 22662, Pierce) at a ratio of 1 mg IDCR per 20 μL reagent.

- Example: Dissolve 60 mg IDCR in 1200 μL of Pierce 660nm Protein Assay reagent for 8 tests.

Step 4: Add 150 μL of the buffer from Step 1-2 (660nm Protein Assay Reagent with IDCR) into each sample well.

Step 5: Incubate the plate for 5 min in the dark at RT and measure the absorbance at 660 nm within 20 min.

Step 6: Before reading the absorbance, mix the samples by pipetting or shaking the plate.

Step 7: Calculate protein concentrations using the standard curve.

- For cells, typically 40–60 μg of total protein can be extracted from a single-well



Note 13: For accurate protein quantification, two steps are important: 1. During cell lysis process, carefully collect the supernatant after sonication and centrifugation. 2. Ensure the supernatant is with protein assay reagent and IDCR by pipetting or shaking the plate before measuring absorbance.

7.3.2. Reagent Set-Up and Preparation for Affinity Purification

Solution	Handling and Preparation	Storage
<u>Dilute (E)</u>	Mix it gently and ensure buffer is transparent.	4°C
<u>Pull (F)</u>	<p>For first-time use:</p> <ol style="list-style-type: none"> 1. Add 200 μL of <u>Dilute (E)</u> into <u>Pull (F)</u> and vortex at 1000 rpm for 5 minutes at RT. 2. Spin down and place diluted <u>Pull (F)</u> on the magnetic rack for 2 min to ensure complete collection of the beads against the tube wall. 3. Remove the supernatant. 4. Gently resuspend the beads with 80 μL of <u>Dilute (E)</u>. <p>For regular use: Vortex to ensure the solution is homogeneous.</p>	4°C
<u>Wash 1 (G)</u>	Mix it gently and ensure buffer is transparent.	4°C
<u>Wash 2 (H)</u>	Mix it gently and ensure buffer is transparent.	4°C
<u>Wash 3 (I)</u>	Mix it gently and ensure buffer is transparent.	4°C
<u>Wash 4 (J)</u>	Mix it gently and ensure buffer is transparent.	4°C

7.3.3. Affinity Purification (Affinity Pull-down, AP)



Step 1: Transfer equal quantities of proteins from unlabeled (UL) or photolabeled (PL) samples into Proteomics tube (CA).



Note 14: Label the tubes as UL or PL. It is recommended to use at least 50 μg for PC samples and 200–400 μg for project samples.

Step 2: Add Dilute (E) to the samples to achieve a five-fold dilution.

- Example: Add 400 μL of Dilute (E) per 100 μL of sample.



Step 3: Retrieve Pull (F), vortex to ensure that the beads are homogeneous. Spin down before use.

Step 4: Homogenize the beads by pipetting and add 10 μL of beads per sample tube.



Note 15: The protein-to-bead ratio ($\mu\text{g}/\mu\text{L}$) and the incubation duration may require optimization based on different targets. Initial testing can be performed using 200–400 μg of protein with 10 μL of beads and an incubation period of 1 hr.

Step 5: Incubate the bead-lysate mixture with end-to-end rotation at RT for 1 hour using ELMI Intelli-Mixer (F1 mode-06).

Step 6: Spin down and place the tubes on a magnetic rack, allow them to stand for 2 min. Then, carefully discard the supernatants, ensuring the beads remain undisturbed.

Step 7: Wash the beads with Wash 1 (G):

Step 7.1: Add 1 mL of Wash 1 (G) into the tubes.

Step 7.2: Vortex the tubes at 1000 rpm for 5 min.

Step 7.3: Spin down and then place the tubes on the magnetic rack for 2 minutes to ensure collection of the beads against the side of the tubes.

Step 7.4: Carefully discard the supernatant, ensuring that the beads remain undisturbed.

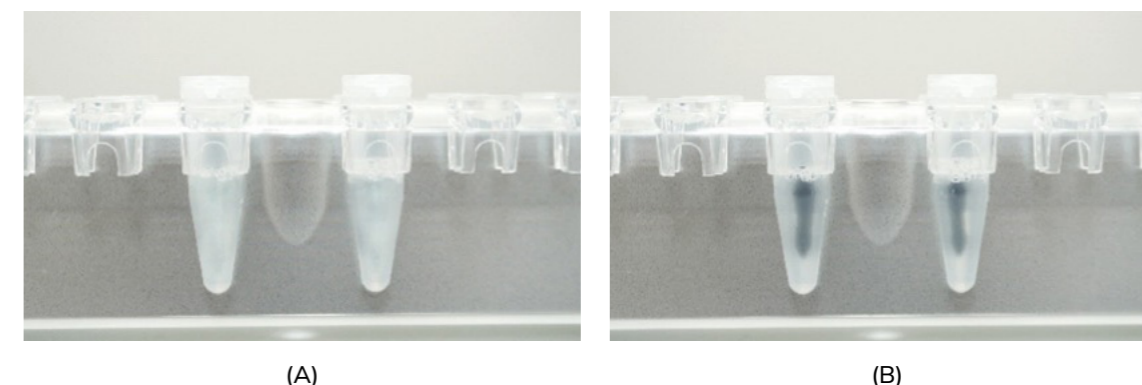


Fig. 7.1 Magnetic beads washing process: before supernatant removal: (A) After vortex (Light blue-black), (B) Ready for supernatant removal (Step 7-3)

Step 8: Repeat Step 7 to wash the beads with Wash 2 (H) and Wash 3 (I).

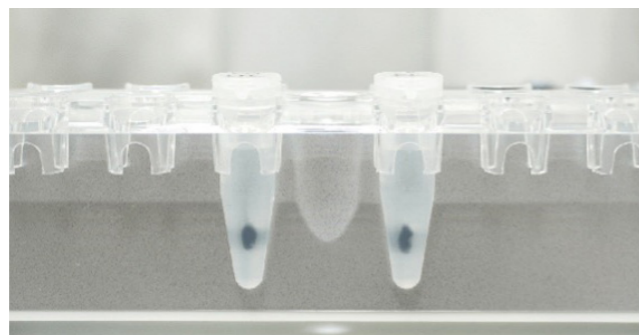
Step 9: Wash the beads with Wash 4 (J):

Step 9.1: Add 1 mL of Wash 4 (J) into the tubes.

Step 9.2: Vortex the tubes at 1000 rpm for 5 min.

Step 9.3: Spin down the beads at 5000 \times g for 3 min.

Step 9.4: Place the tubes on the magnetic rack for 2 minutes to ensure complete beads collection as fig 7.2.

Fig. 7.2 Complete beads collection after Wash 4 (J) and centrifugation

Step 9.5: Carefully discard the supernatant, ensuring that the beads remain undisturbed.

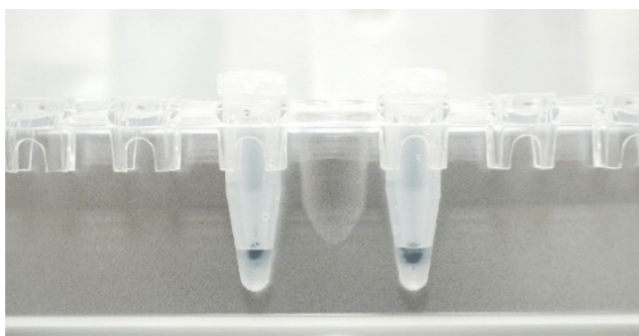
Step 10: Add 100 μ L of Wash 4 (J) into the tubes and sonicate for 10 seconds in an ultrasonic water bath.

Please ensure the solution is homogenous and exhibits a uniform blue-black color as shown in Fig 7.1 (A).

Step 11: Spin down and then place the tubes on the magnetic rack for 2 min to ensure complete adhesion of the beads to the magnetic rack/surface.

Step 12: Carefully discard the supernatant, ensuring that the beads remain undisturbed and intact.

Step 13: Repeat Steps 10-12 for a total of 3 times washes.

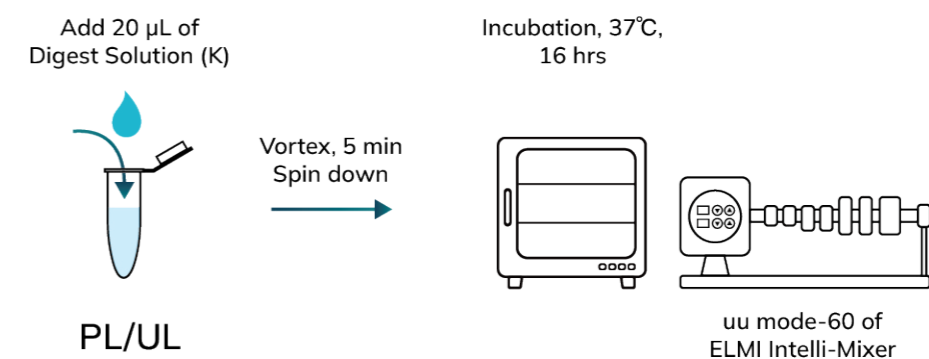
Fig. 7.3 Beads adhesion after 3 washes with Wash 4 (J).

7.3.4 Reagent Set-Up and Preparation for On-Bead Digestion

Solution	Handling and Preparation	Storage
<u>Wash 4 (J)</u>	Mix it gently and ensure buffer is transparent.	4°C
<u>Digest Solution (K)</u>	For first-time use: Spin down before opening, dissolve <u>Digest (K)</u> in 150 μ L of <u>Wash 4 (J)</u> , then vortex, spin down and keep at 4°C (or on ice). For regular use: Vortex to ensure the solution is homogeneous, spin down and keep at 4°C (or on ice).	-20°C

7.3.5 On-Bead Digestion

Overview



Step 1: Briefly spin down the Digest Solution (K), remove the tubes from the magnetic rack, then resuspend the beads in 20 μ L of Digest Solution (K) for each sample (UL and PL).

Note 16: Return Digest Solution (K) to -20°C after use.

Step 2: Incubate the samples at 37°C for 16 hours with side-to-side shaking using the ELMI Intelli-Mixer (uu mode-60).

Ensure the beads are homogeneous, with no precipitates and no splashing.

Stopping Point

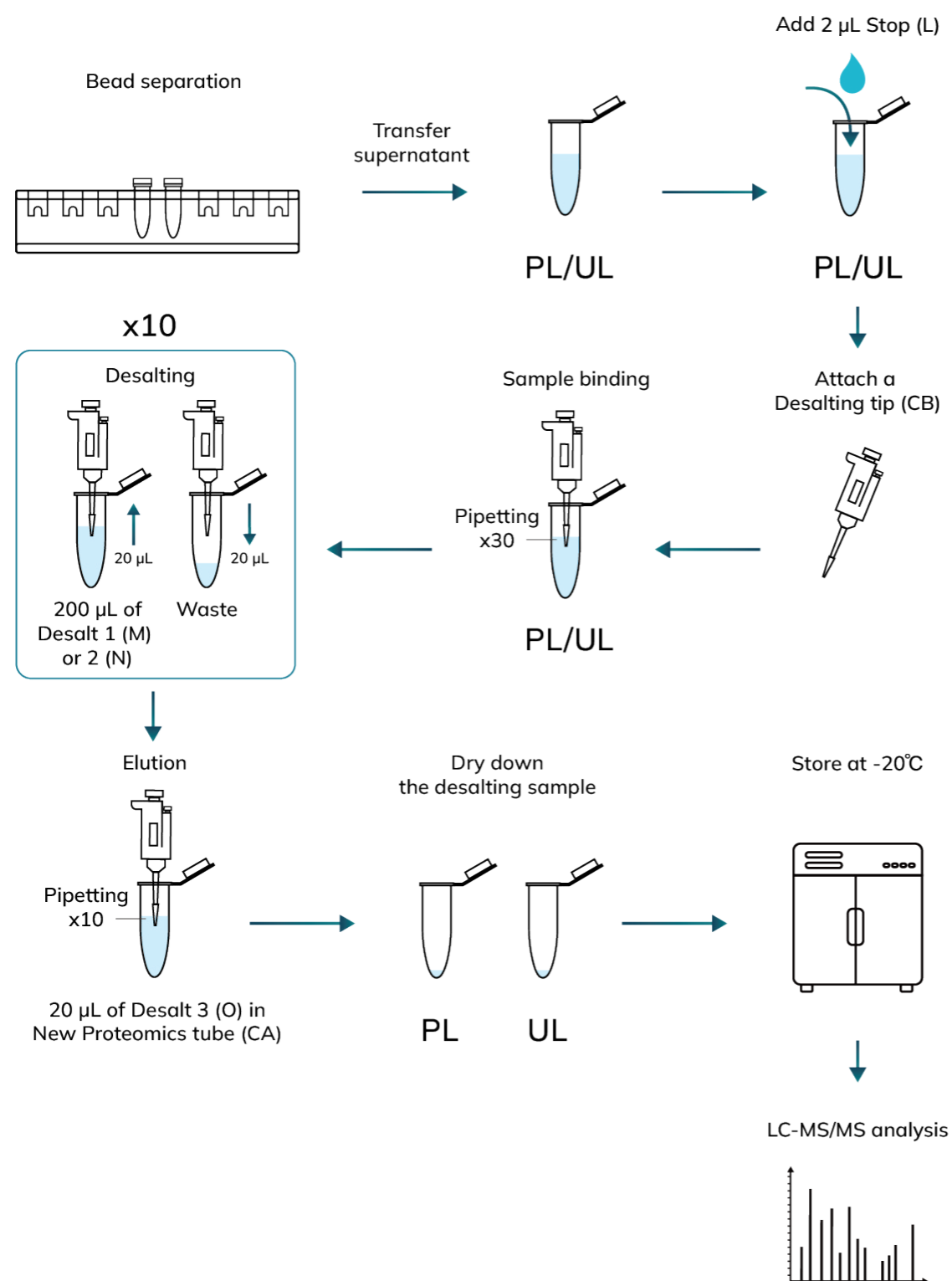
After this step, proceed with peptide desalting for LC-MS/MS analysis.

7.4 Peptide Desalting

7.4.1 Reagent Set-Up and Preparation

Solution	Handling and Preparation	Storage
<u>Stop (L)</u>	Vortex and spin down.	4°C
<u>Desalt 1 (M)</u>	Add 200 μ L of <u>Desalt 1 (M)</u> into a new 1.5-mL tube labeled as "Desalt 1" per sample.	4°C
<u>Desalt 2 (N)</u>	Add 200 μ L of <u>Desalt 2 (N)</u> into a new 1.5-mL tube labeled as "Desalt 2" per sample.	4°C
<u>Desalt 3 (O)</u>	Add 20 μ L of <u>Desalt 3 (O)</u> into a new Proteomics tube (CA) labeled with the name of your specific project sample.	4°C

Overview



Step 1: Separate the beads from the sample by spinning down and placing the tubes on the magnetic rack for 30 seconds.

Step 2: Transfer the supernatants to new Proteomics tubes (CA) (UL and PL) without disturbing the beads.

Step 3: Add 2 µL of Stop (L) to each sample, vortex and spin down.

Step 4: Attach a Desalting tip (CB) to a P20 Pipette

Step 5: Procedures for desalting:

Step 5.1: Binding step:

- Place the Desalting tip (CB) into the sample (from Step 3).
- Perform 30 aspirate–dispense cycles.

Step 5.2: Desalting step:

- Place the same Desalting tip (CB) in Desalt 1.
- Aspirate 20 µL of Desalt 1, then dispense it as waste.
- Repeat 10 times.

Step 5.3: Wash step:

- Place the same Desalting tip (CB) in Desalt 2.
- Aspirate 20 µL of Desalt 2, then dispense it as waste.
- Repeat 10 times.

Step 5.4: Elution step:

- Place the same Desalting tip (CB) in Desalt 3.
- Perform 10 aspirate–dispense cycles.

Step 5.5: Discard the used Desalting tip (CB) after completing the Elution step.

Step 6: To desalt additional samples, repeat steps 5.1-5.5 using a new Desalting Tip (CB) for each sample.

Step 7: Dry down the desalted samples from Step 5.4 using a SpeedVac concentrator for 2 hours at RT.

Step 8: Store the dried samples at -20°C until LC-MS/MS analysis.

End Point



This marks the end of pull-down procedures. Dried samples can be stored at -20°C for up to 1 month before performing LC-MS/MS analysis.



Note 17: For optimal tryptic peptide analysis using the Synpull™ Kit, it is recommended to use a nanoscale liquid chromatography system coupled with a mid-end to high-end mass spectrometer (MS). When targeted structures are particularly small or sparse, a mass spectrometer with an MS/MS acquisition rate above 20 Hz is advised.

Examples of suitable mass spectrometers include:

- Orbitrap™ series (Exploris Fusion™ Lumos™, Eclipse™ and Astral™) (Thermo Fisher Scientific)
- timsTOF series (SCP, Pro 2, HT, Ultra, Ultra 2) (Bruker)
- TripleTOF® and ZenoTOF (SCIEX).



Note 18: After purification and drying, an on-line C18 trapping column or off-line desalting procedure by ZipTip with C18 resin (e.g., CATALOG NUMBER: ZTC18S096, Merck Millipore) is necessary for further LC-MS/MS analysis.

7.5 Identification of the Proteome from Regions of Interest

Step 1: Both label-free quantitation and isobaric labeling quantitation approaches are compatible with samples processed using the Synpull™ Kit.

- For isobaric labeling quantitation, proceed with isobaric labeling using commercial products such as the iTRAQ™ or TMT™ kit.

Step 2: Resuspend the desalted samples in an appropriate volume of 0.1% formic acid (FA) in water.

Sample type	Amount of 0.1% FA/water	Injection volume	No. of MS Analysis
Positive Control Sample	7 µL	5 µL	Single LC-MS/MS analysis
Specific Project Sample	12 µL	5 µL	Duplicated LC-MS/MS analysis

* The suggested injection volume was tested using the Orbitrap™ Fusion™ Lumos™ MS. For high-end mass spectrometer (MS) (e.g., Orbitrap™ Astral™, timsTOF Ultra 2), the injection volume can be reduced up to 10 times.

Step 3: Perform 20-30 dispense–aspirate cycles to rinse the tube wall from 50-µL height to bottom.

Step 4: After centrifugation at 16000 × g for 2 min at RT, transfer the supernatant to the sample vial for LC-MS/MS analysis and ensure no air bubbles remain at the vial bottom.

Step 5: Analyze the resuspended samples using LC-MS/MS with an suitable gradient. A LC separation time of 120 min is required for PC samples.

Time (min)	Flow rate (µL/min)	Solvent A (0.1% FA/Water)	Solvent B (0.1% FA/ACN)
0	0.3	98%	2%
2	0.3	96%	4%
85	0.3	80%	20%
108	0.3	62%	38%
109	0.3	5%	95%
113	0.3	5%	95%
114	0.3	98%	2%
120	0.3	98%	2%

The provided chromatogram in Fig. 7.4 illustrates a typical TIC chromatogram of the Positive Control sample using a 2-hour gradient on an Orbitrap Fusion Lumos mass spectrometer operating in DDA mode.

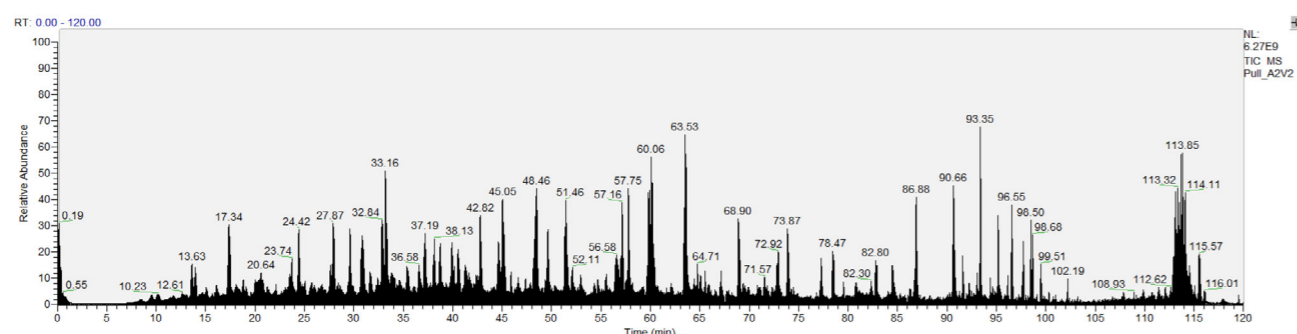


Fig. 7.4 The chromatogram of the Microscop® nuclear proteins digest standard.

Step 6: Use software tools such as DIA-NN, MaxQuant, Proteome Discoverer™, Skyline, or Spectronaut® for protein identification and quantitation. Set the following parameters:

- Static modification: carbamidomethylation (+57.0215 Da) on Cys residues.
- Dynamic modification: deamidation (+0.9840 Da) on Asn and Gln residues, oxidation (+15.9949 Da) on Met residues, acetylation on protein N-termini (+42.0106 Da).
- Enzyme: trypsin.
- Enable imputation for missing values.

Step 7: Remove contaminant proteins (e.g., from MaxQuant at <https://maxquant.org/maxquant/> or Universal Protein Contaminant Libraries at https://pubs.acs.org/doi/suppl/10.1021/acs.jproteome.2c00145/suppl_file/pr2c00145_si_004.txt).

Step 8: For PC samples, rank proteins based on the abundance ratio (PC-PL/PC-UL). Submit the top 20% of proteins to GO Enrichment Analysis to verify the nuclear specificity:

Step 8.1: Access GO Enrichment Analysis at <https://www.geneontology.org/>.

Step 8.2: Paste the gene names or Uniprot IDs.

Step 8.3: Choose “cellular component” and “Homo sapiens”.

Step 8.4: click “Launch”.

Step 8.5: Check that the results of the PC samples meet the following criteria:

- Sensitivity: the total number of identified proteins is expected to exceed 500.
- Specificity: the top 20% of proteins should exhibit over 80% nuclear specificity (number of nucleus protein/Uniquely Mapped IDs).

Step 9: For specific projects, analyze the mass spectrometric data using statistical tools such as ratio ranking, volcano plots, and Receiver Operating Characteristic (ROC) curves. Here demonstrate the data visualization using a volcano plot:

Step 9.1: Calculate the abundance ratio of PL to UL samples and transform to the log₂ (fold-change).

Step 9.2: Calculate the p-value from the duplicates of the abundance values for PL and UL samples. and transform to the -log₁₀ (p-value).

Step 9.3: Organize the protein list as in the table below:

Uniprot ID	Description	Gene	Log ₂ (fold-change)	-Log(p-value)	#Unique Peptides
1233	Cell Function	A	10.15	1.68	2
1234	Cell Function	B	2.97	1.74	1
1235	Cell Function	C	2.93	1.60	2
1236	Cell Function	D	2.86	1.80	2
1237	Cell Function	E	2.55	1.33	2

Step 9.4: Use \log_2 (fold-change) as X axis, and $-\log_{10}$ (p-value) as Y axis to create a plot to visualize the enrichment efficiency.

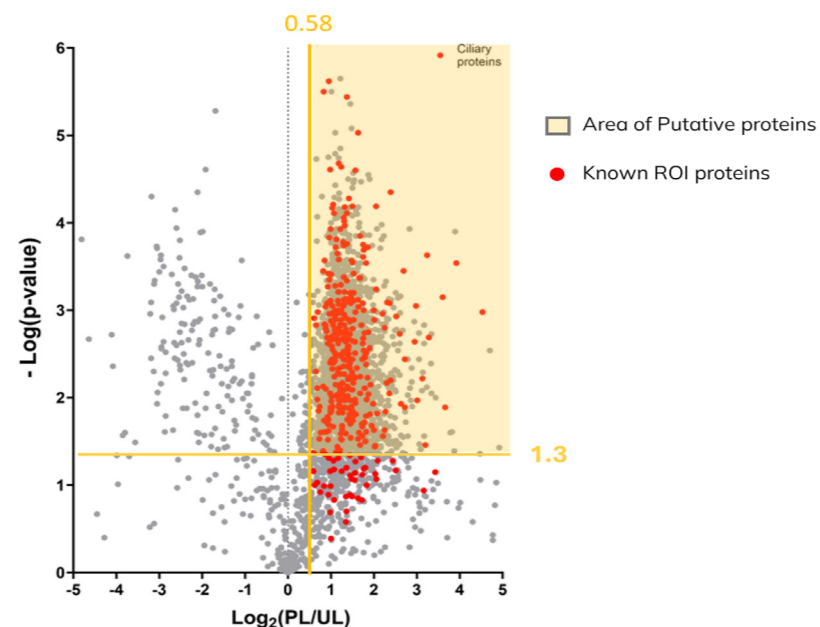


Fig. 7.5 Example of volcano plot



Note 19: Identification of putative proteins

Proteins that meet the following criteria are putative proteins from ROIs. Users can set the filters based on experimental needs.

Standard confidence:

- \log_2 (fold-change > 1.5) > 0.58

High confidence:

- \log_2 (fold-change > 1.5) > 0.58
- $-\log_{10}$ (p-value < 0.05) > 1.3
- Number of unique peptides > 1

Priority validation with high confidence:

- TOP10% ratio ranked (PL/UL) proteins

Step 9.5: Identify the potential novel proteins for downstream analysis based on prior knowledge and bioinformatics analysis.

8. APPENDIX: TROUBLESHOOTING

Troubleshooting Guide			
Part	Problem	Possible Cause	Recommended Action
Lysis	Low protein yield from cells	Aggregation of cell pellet	Extend sonication time to minimize the amount of visible pellet.
	Low protein yield from tissue	Aggregation of tissue pellet	Extend sonication time to minimize the amount of visible pellet.
Sonication	Low protein yield	Too strong sonication causing the sample to splash	Use an ultrasonic water bath for cell samples and optimize sonication intensity for tissue samples.

Protein assay	Precipitate formation	Sample has been incubated beyond the recommended time	Shake the plate gently to mix the sample thoroughly before measuring absorbance. Avoid incubation periods exceeding 20 minutes.
Affinity purification	Low protein binding	High detergent concentration interferes with protein binding	Ensure Dilute (E) has been added to the samples to achieve a five-fold dilution.
	Low protein sensitivity and abnormal liquid chromatography	Residual detergent and salt interfere with protein binding	Add Wash 4 (J) (100 μ L at a time) to wash beads until the beads appear thin and sticky against the magnetic rack as shown in Fig. 7.3.
Digestion	Low protein sensitivity	Missed cleavage of peptide > 25%	Confirm Digest (K) has been stored at -20°C and has not been freeze-thawed more than three times. Ensure the sample was digested with Digest Solution (K) for at least 16 hours.
	Low protein sensitivity	Beads precipitated during on-bead digestion	Ensure the beads are well-mixed without splashing and resuspended in the Digest Solution (K).
Desalting	Low protein sensitivity	Buffer condition/ order is incorrect	After adding Stop (L), confirm sample pH < 4. Confirm the buffers and order of the desalting steps.
LC-MS/MS	Low protein sensitivity	LC separation time is too short; Low-end mass spectrometer (MS) used	Set the separation time to 120 min for PC samples; Use the MS with MS/MS acquisition rate above 20 Hz.
	Low specificity of ROI proteins	Low labeling amount (Pixel counts < 1×10^8 from Microscoop [®]). Low labeling efficiency	Prepare new samples to achieve higher labeling amount and efficiency. Ensure each IFU note described in Synpull [™] Kit has been met.
LC-MS/MS	Target/well-known proteins not identified	Target selected may be a low-abundance protein	Increase number of samples/slides and areas for labeling (Pixel counts), use data-independent acquisition (DIA) approach for the detection of low-abundance proteins.

9. PURCHASER NOTIFICATION

9.1 Contact Information:

Manufacturer: SYNCELL (TAIWAN) Inc.

Email: Info@syncell.com **Tel:** +886-2-2785-6780

Address: 14F, No. 508, Sec. 7, Zhongxiao E. Rd., Nangang Dist., Taipei City 115, Taiwan

European Authorized Representative: Luana Med B.V.

Address: Abtswoudseweg 18, 2627AL, Delft, NL.

9.2 Customer Support:

For the latest services and support information for all locations, go to customerservice@syncell.com.

On the website, you can












- search through frequently asked questions (FAQs)
- submit a question directly to Technical Support

Safety data sheets (SDSs) are available at the QR code below:



For research use only (RUO), not for use in diagnostic procedures.

9.3 Symbols

Symbols			
	Catalog number		Temperature limit
	Batch code		Indicates the total number of tests that can be performed with the product
	Date of manufacture		Consult instructions for use
	Use by date		Storage condition (avoid light): keep away from sunlight
	Manufacturer		Authorized representative in the European Community/ European Union
	CE marking		

**For the latest services and support information for all locations,
go to <https://www.syncell.com/>.**

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