# Recommendations for Specific Sample Types

***Protein concentration should be assayed by BCA for all samples except immunoprecipitations (IPs).***

| Sample Type | Recommendation | Reference |
|---|---|---|
| **Adherent Cells** | Wash cells with PBS. Lyse with ProtiFi™ 2x Lysis Solution containing protease inhibitor, 0.1% Benzonase, and phosphatase inhibitor cocktail for 30 minutes on ice. Scrape cells, transfer to Eppendorf tubes, and centrifuge at 21,000 x g for 15 minutes at 4°C. Collect the supernatant, determine the protein concentration, and bring the SDS concentration to 5% prior to S-Trap™ digestion. | Ahn, G., et al. *Elucidating the cellular determinants of targeted membrane protein degradation by lysosome-targeting chimeras.* Science 382, 6668 (2023). https://doi/10.1126/science.adf6249 |
| **Bacteria and Archaebacteria** | Pellet cells and wash three times with PBS solution. To facilitate lysis and protein extraction, resuspend samples in PBS containing 100 µg/mL lysostaphin, 100 U/mL DNAsel, 25 U/mL RNAsel, and 2x EDTA free protease inhibitor cocktail. Incubate for 30 minutes at 37 °C. Bead beat using silica beads; five rounds of bead beating for 45 seconds each, with 1 minute rest intervals in between on ice. Centrifuge 17,000 x g for 10 minutes. Collect soluble fraction and insoluble fraction. Wash the insoluble fraction with PBS and centrifuge at 17,000 x g for 5 minutes. Proceed with S-Trap™ digestion for the different fractions, solubilizing in ProtiFi™ 2x Lysis Solution. | Mustor, E. M., et al. *A Simplified Method for Comprehensive Capture of the Staphylococcus aureus Proteome.* bioRxiv (2024). https://doi.org/10.1101/2024.08.07.607079 |
| **Bile** | Mix bile protein precipitate 1:1 with ProtiFi™ 2x Lysis Solution and proceed with S-Trap™ digestion. | Thorne, A. M., et al. *Comparative Analysis of Digestion Methods for Bile Proteomics: The Key to Unlocking Biliary Biomarker Potential.* Anal. Chem. 96 (36): 14393–14404 (2024). https://doi.org/10.1021/acs.analchem.4c01766 |
| **Bioreactor Supernatant** | Mix bioreactor supernatant 1:1 with ProtiFi™ 2x Lysis Solution and proceed with S-Trap™ digestion. Polymeric surfactants such as Pluronic F68 will be fully removed. | Zacchi, L. F., et al. *S-Trap Eliminates Cell Culture Media Polymeric Surfactants for Effective Proteomic Analysis of Mammalian Cell Bioreactor Supernatants.* J. Proteome Res. 19 (5): 2149-2158 (2020). https://doi.org/10.1021/acs.jproteome.0c00106 |
| **COVID Nasopharyngeal Swabs** | Collect nasopharyngeal swabs in 500 µL of Viral Transport Media. Remove swabs. Inactivate by diluting 1:1 with ProtiFi™ 2x Lysis Solution. Incubate on an end over end rotar for 20 minutes at room temperature. To each sample, add TCA to 10% from a 50% w/v stock to concentrate samples. Mix samples thoroughly. Centrifuge at 12,000 rpm at 4 °C for 10 minutes. Discard the supernatant and wash pellet three times with cold acetone. Resuspend with ProtiFi™ 1x Lysis Solution and continue with S-Trap™ digestion. | Pinto, G., et al. *Identification of SARS-CoV-2 Proteins from Nasopharyngeal Swabs Probed by Multiple Reaction Monitoring Tandem Mass Spectrometry.* ACS Omega 6 (50): 34945-34953 (2021). https://doi.org/10.1021/acsomega.1c05587 |
| **Exosomes (EVs)** | Isolate EVs with preferred method; see reference for thorough evaluation. Digest EV samples using a modified S-Trap™ protein digestion protocol. Briefly, mix EVs (equivalent to 3 µL of plasma-derived EVs, diluted up to 50 µL in PBS) with 50 µL of ProtiFi™ 2x Lysis Solution and sonicate for 10 minutes. Add 2 µL of ProtiFi™ Reductant and incubate at 55 °C for 20 minutes. Add 2 µL of ProtiFi™ Alkylator and incubate at room temperature for 20 minutes. Add 5 µL of acidifier (30% phosphoric acid), vortex. Add 200 µL of ProtiFi™ Binding/Wash Solution and load on a S-Trap™ micro column. | Suresh, P. S., and Zhang, Q. *Comprehensive Comparison of Methods for Isolation of Extracellular Vesicles from Human Plasma*. J. Proteome Res. (2025). https://doi.org/10.1021/acs.jproteome.5c00149 |
| **Formalin-Fixed Paraffin-Embedded (FFPE) Samples - Ultrasonication** | Follow the HYPERsol protocol with the following alteration: allow cores or scrolls to hydrate in ProtiFi™ 1x Lysis Solution overnight. The next day, sonicate for 5 minutes or until pieces have been dissolved. Proceed with S-Trap™ digestion. If the PIXUL is to be utilized, refer to HYPERsol PIXUL protocol 2.2. | Marchione, D. M., et al. *HYPERsol: High-Quality Data from Archival FFPE Tissue for Clinical Proteomics.* J. Proteome Res. 19 (2): 973-983 (2020). https://doi.org/10.1021/acs.jproteome.9b00686 |

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|---|---|---|
| **FFPE Samples – Bead Beating** | Collect 4 µm FFPE sections in 2 mL tubes. Add three 1 mm zirconium beads. Add 125 µL of xylene and deparaffinize with three 30 second cycles on a bead beater. Remove xylenes and add 125 µL of ProtiFi™ 2x Lysis Solution. Vortex for 15 minutes at room temperature to dissolve samples. Reduce using ProtiFi™ Reductant and alkylate with ProtiFi™ Alkylator. Acidify with 25 µL of 12% phosphoric acid. Add 900 µL ProtiFi™ Binding/Wash Solution and bind on an S-Trap™ 96-well plate, sequentially add and bind 400 µL of the sample mixed with ProtiFi™ Binding/Wash Solution until all sample have been passed through the plate. Wash captured proteins once with 400 µL of 50% chloroform/50% methanol and 5 times with 400 µL of ProtiFi™ Binding/Wash Solution. Proceed with S-Trap™ digestion. | Beddows, I., et al. *Impact of BRCA mutations, age, surgical indication, and hormone status on the molecular phenotype of the human Fallopian tube.* Nat. Commun. 16, 2981 (2025). https://doi.org/10.1038/s41467-025-58145-2 |
| **Immunoprecipitations (IPs)** | Post-IP, wash beads three times with an appropriate wash solution (ex. 20 mM Tris-HCl pH 7.5, 200 mM NaCl, 5 mM $MgCl_2$, 0.2% Triton, and 1 mM DTT). Elute with ProtiFi™ 2x Lysis Solution (100 mM Tris-HCl pH 7.5, 10% SDS). Follow the S-Trap™ protocol, wash six times with ProtiFi™ Binding/Wash Solution. | Tagnères, S. et al. *SURF2 is a MDM2 antagonist in triggering the nucleolar stress response*. Nat. Commun. 15, 8404 (2024) https:\\doi.org\10.1038/s41467-024-52659-x |
| **Membrane Fractions / Hydrophobic Proteins – Density Gradient** | Isolate membrane fraction by sonicating. And, if desired, enrich with OptiPrep or ultracentrifugation. If desired, concentrate with 10% TCA, wash with cold ethanol four times, pellet, and proceed with S-Trap™ digestion. Otherwise, proceed to normal S-Trap™ digestion. | Chhuon, C., et al. *A sensitive S-Trap-based approach to the analysis of T cell lipid raft proteome.* J. Lipid Res. 61 (11): 1512–1523 (2020). https://doi.org/10.1194/jlr.D120000672 |
| **Membrane Fractions / Hydrophobic Proteins – Membrane Labeling** | Perform surface biotinylation followed by streptavidin bead enrichment. After the final wash, resuspend in ProtiFi™ 2x Lysis Solution containing 20 mM DTT and 25 mM biotin. Heat to 95 °C for 10 minutes. Transfer supernatant to a new sample tube. Proceed with S-Trap™ digestion. | Floyd, B. M., et al. *Mapping the nanoscale organization of the human cell surface proteome reveals new functional associations and surface antigen clusters.* bioRxiv (2025). https://doi.org/10.1101/2025.02.12.637979 |
| **Cells** | Wash cells with PBS three times. For adherent cells, lift cells using a cell scraper. Centrifuge at 500 RCF for 5 minutes, pellet, and store at -80 °C until use. Lyse frozen cell pellets in ProtiFi™ 2x Lysis Solution. If desired, shear DNA with sonication or Benzonase®. Centrifuge sheared cells at 13,000 RCF for 10 minutes, and retain protein supernatant. Proceed with S-Trap™ digestion. | Shannon, A. E., et al. *Rapid assay development for low input targeted proteomics using a versatile linear ion trap*. Nat. Commun. 16, 3794 (2025). https://doi.org/10.1038/s41467-025-58757-8 |
| **Peripheral Blood Mononuclear Cells (PBMCs)** | Homogenize cells using bead beating, then lyse cells in ProtiFi™ 1x Lysis Solution. Sonicate and proceed with S-Trap™ digestion. | Kra, G., et al. *Proteome dataset of peripheral blood mononuclear cells in postpartum dairy cows supplemented with different sources of omega-3 fatty acids,* Data in Brief (40) (2022). https://doi.org/10.1016/j.dib.2021.107785 |
| **Saliva** | Cryopreserved (– 80 °C) saliva samples are inactivated, reduced, and alkylated with a 1:1 v/v of 2x denaturing solution (10% SDS, 200 mM TEAB, 10 mM TCEP, and 10 mM CAA), followed by incubation at 60 °C for 30 minutes. Proceed with S-Trap™ digestion. | Moreno, E., et al. *Proteomic snapshot of saliva samples predicts new pathways implicated in SARS-CoV-2 pathogenesis.* Clin. Proteom. 21, 37 (2024). https://doi.org/10.1186/s12014-024-09482-9 |
| **Serum / Plasma** | Denature sample in ProtiFi™ 1x Lysis Solution and proceed with S-Trap™ digestion. | Mindikoglu, A. L., et al. *Intermittent fasting from dawn to sunset for 30 consecutive days is associated with anticancer proteomic signature and upregulates key regulatory proteins of glucose and lipid metabolism, circadian clock, DNA repair, cytoskeleton remodeling, immune system and cognitive function in healthy subjects.* J. Proteomics 217, 103645 (2020). https://doi.org/10.1016/j.jprot.2020.103645 |
| **Stool Samples** | Dissolve stool with ProtiFi™ 1x Lysis Solution. Incubate at 96 °C for 5 minutes, followed by six cycles of 30 second sonication. Proceed with S-Trap™ digestion. | Valdés-Mas, R. , et al. *Metagenome-informed metaproteomics of the human gut microbiome, host, and dietary exposome uncovers signatures of health and inflammatory bowel disease*. Cell 188(4): 1062-1083.E36 (2025). https://doi.org/10.1016/j.cell.2024.12.016 |

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|---|---|---|
| **Tissue Samples** | Dissect out ~1 mg tissue and freeze immediately with a dry ice bath or liquid nitrogen. Transfer frozen tissue to a 1.0 mL homogenizer. Add 100 µL (or desired volume; aim for 3- 10x w/v tissue weight:ProtiFi™ 1x Lysis Solution) of ProtiFi™ 1x Lysis Solution. Homogenize at 4 °C. Transfer homogenized tissue in lysis solution to a sample tube and boil for 2 minutes. Centrifuge the sample for 10 minutes at 14,000 x g at room temperature, then collect the supernatant and proceed with S-Trap™ digestion. Pellets can be analyzed separately.<br>Bone will require demineralization with EDTA; larger pieces may require days. Length and power of sonication must be optimized for each tissue. | Hu, M. and Wang, Y. *Optimized Workflow for Proteomics and Phosphoproteomics With Limited Tissue Samples.* Current Protocols 4: 4 (2024). https://doi.org/10.1002/cpz1.1028 |
| **Urine** | Aliquot 0.5 mL urine and add 2 mL of cold acetone. Precipitate the proteins overnight at −20 °C. Pellet, then dissolve in ProtiFi™ 1x Lysis Solution. Proceed with S-Trap™ digestion. Refer to Urine S-Trap protocol 1.2 for a complete protocol. | Ding, H., et al. *Urine Proteomics: Evaluation of Different Sample Preparation Workflows for Quantitative, Reproducible, and Improved Depth of Analysis* J. Proteome Res. 19 (4), 1857-1862 (2020). https://doi.org/10.1021/acs.jproteome.9b00772 |
| **Yeast** | Collect yeast cells using 5 mL of 10% w/v ice-cold TCA. Centrifuge cells at 1,000 x g for 2 minutes at 4 °C. Discard supernatant. Wash cells first in 5 mL of acetone (cooled down at −20 °C) and then in 1 mL of lysis solution containing 10 mM DTT. Resuspend pellets in 400 µL of ProtiFi™ 1x Lysis Solution. Add acid-washed glass beads (Sigma; G8772) and lyse cells using a FastPrep-24 5G bead beating grinder (six times shaking at 100 V for 30 seconds, 30 second break between runs). Centrifuge at maximal speed for 5 minutes at 4 °C and snap freeze the supernatant. For processing, samples are heated at 95 °C for 10 minutes with shaking to lyse cells. Proceed with S-Trap™ digestion. | Bérard, M., et al. *Proteomic and phosphoproteomic analyses reveal that TORC1 is reactivated by pheromone signaling during sexual reproduction in fission yeast.* PLoS Biol. 22 (12): e3002963(2024). https://doi.org/10.1371/journal.pbio.3002963 |