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 S-Trap™ Solution 1, 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; ideally cryopulverize the sample (e.g. Retsch CryoMill) or alternatively bead beat using silica beads; five rounds of bead beating for 45 seconds each, with 1 minute rest intervals in between on ice. Warm/melt (if applicable) and incubate for 30 minutes at 37 °C; sonication will improve results. 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 S-Trap™ Solution 1, 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 S-Trap™ Solution 1, 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 S-Trap™ Solution 1, 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 S-Trap™ Solution 1, 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 S-Trap™ 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 S-Trap™ Solution 1, 2x Lysis Solution and sonicate for 10 minutes. Add 2 µL of S-Trap™ Solution 2, Reductant and incubate at 55 °C for 20 minutes. Add 2 µL of S-Trap™ Solution 3, Alkylator and incubate at room temperature for 20 minutes. Add 5 µL of acidifier (30% phosphoric acid), vortex. Add 200 µL of S-Trap™ Solution 5, Binding/Wash Solution and load on a S-Trap™ Micro Spin 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 FFPE with PIXUL protocol with the following alteration: Sonicate for 5 minutes or until pieces have been dissolved using the available sonicator in place of the PIXUL megasonicator.	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

Sample Type	Recommendation	Reference
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 S-Trap™ Solution 1, 2x Lysis Solution. Vortex for 15 minutes at room temperature to dissolve samples. Reduce using S-Trap™ Solution 2, Reductant and alkylate with S-Trap™ Solution 3, Alkylator. Acidify with 25 µL of 12% phosphoric acid. Add 900 µL S-Trap™ Solution 5, Binding/Wash Solution and bind on an S-Trap™ 96-well Mini, sequentially add and bind 400 µL of the sample mixed with S-Trap™ Solution 5, 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 S-Trap™ Solution 5, 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 S-Trap™ Solution 1, 2x Lysis Solution (100 mM Tris-HCl pH 7.5, 10% SDS). Follow the S-Trap™ protocol, wash six times with S-Trap™ Solution 5, 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 S-Trap™ Solution 1, 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 S-Trap™ Solution 1, 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 S-Trap™ 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 S-Trap™ 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 S-Trap™ 1x Lysis Solution. Incubate at 96 °C for 5 minutes, followed by six cycles of 30 second sonication. Proceed with a protein assay and standard 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

Sample Type	Recommendation	Reference
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: S-Trap™ 1x Lysis Solution) of S-Trap™ 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. Bone will require demineralization with EDTA; larger pieces may require days. Length and power of sonication must be optimized for each tissue. Pellets can be analyzed separately and note that they are NOT necessarily uninteresting, simply less soluble. Analyze pellets separately by making a suspension, performing all standard S-Trap™ steps and loading the suspension onto an S-Trap™.	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 S-Trap™ 1x Lysis Solution. Proceed with S-Trap™ digestion.	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 S-Trap™ 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