TECHNICAL NOTE

Unlock Unprecedented Depth in Cellular Proteomics with Proteograph™ XT

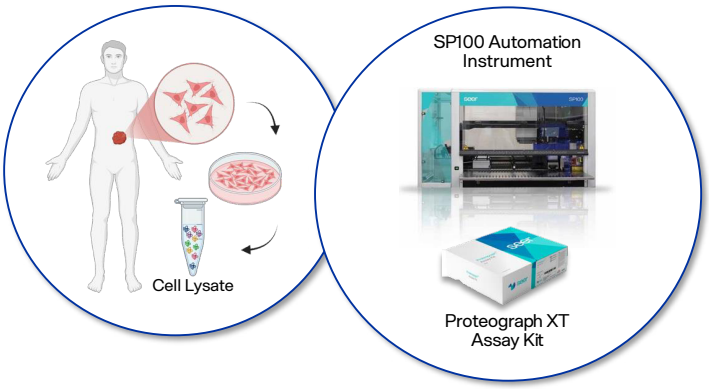
Maedeh Zamani, Adam Brewer, Ambika Sundaresan, Shao-Yung Chen, Xiaoyan Zhao, Ryan Benz, Aaron S. Gajadhar

Introduction

In vitro cell culture of primary cells and cell lines is an invaluable tool in life science research, offering reproducibility, accessibility, and versatility across various applications including basic research and drug discovery. Cell lines have contributed to numerous scientific breakthroughs, including vaccine development and informing our fundamental understanding of cancer and other disease states. Key advantages of cell lines include their cost-effectiveness compared to primary cells or animal models as well as providing a virtually unlimited supply of experimental material in a consistent and reproducible manner. Cell lines are relatively easy to generate and use for large-scale or high-throughput screening studies. They are widely used in pharmaceutical and drug discovery research serving as in vitro tools for screening of potential drug candidates, studying drug response and mechanism of action, and even predicting clinical responses to targeted therapies. Recent advancements in genome editing technology, synthetic biology platforms and 3D cell culture techniques have further enhanced their utility.

Highlights

- Proteograph™ XT nanoparticle technology enables deep cellular proteome analysis capable of measuring over 11,000 proteins.
- High-value proteins across diverse cellular compartments, functions, and structures are captured more extensively with Proteograph XT.
- Proteograph XT probes the full range of cellular proteome abundance, from tens to millions of protein copies per cell.
- Proteograph XT standardized cell lysis workflow with automated sample processing ensures high measurement reproducibility for highly reliable downstream analysis.



Mass spectrometry (MS)-based proteomics of cell lines has matured into a powerful tool for biological discovery complementing analysis of clinical biofluids like blood plasma and other patient biospecimens. MS workflows can provide systems-level, direct measurements of protein abundance, modifications, and interactions thereby complementing genomic, transcriptomic, and metabolic approaches. Mass spectrometry-based proteomic characterization methods of cell lines have advanced significantly in recent years, enabling deeper proteome coverage, higher throughput, and increased sensitivity. Current state-of-the-art approaches can identify and quantify thousands of proteins from single cells and provide comprehensive proteomic profiles of widely used cell line models.

Despite recent advancements in sample preparation techniques and mass spectrometry instrumentation for cellular proteomics, these advancements are not readily accessible to all researchers due to their time-intensive nature, propensity to introduce variability, and frequent demand for a substantial degree of technical expertise. Additionally, traditional proteomic workflows often struggle to detect and quantify low-abundance proteins in cell lines where wide dynamic range of protein abundances in cells is commonly observed. Lastly, the measurement of proteins in sub-cellular compartments like the nucleus and cell membrane is often problematic due to inefficient protein extraction protocols that limit their detection, particularly with hydrophobic targets like membrane proteins.

In this technical note, we present an experimental protocol for reproducible, comprehensive and sensitive cellular proteome analysis using a simplified cell lysis procedure combined with the standard Proteograph XT Assay Kit and SP100 automation instrument. Using proprietary engineered nanoparticle (NP) technology and automated sample preparation, the vast dynamic range of protein composition is characterized with high fidelity, allowing unbiased exploration and quantification of the proteomic landscape in cell line samples.

An evaluation of cell lysis protocols was conducted using a panel of cell lysis buffers, cell input concentrations, and lysis methodologies to determine a sanctioned workflow for Proteograph XT cell lysate analysis. Our findings informed the development of a recommended cell lysis analysis protocol demonstrating routine detection of 10,000–11,000 proteins in both HeLa and HEK293 cell lines with a 10% boost in proteome coverage and over 20% more unique peptides, compared to conventional MS-based cell lysate analysis. Notably, the Proteograph XT nanoparticle workflow enhanced the capture of key protein targets, including nuclear, cell membrane, and DNA-binding proteins, as well as kinases and receptors – all critical players in cellular signaling, gene regulation, and disease pathways. Impressively, a >40% detection improvement was observed for low-abundant proteins of <500 copies/cell in both tested cell lines. High quantitative consistency was observed with proteome-wide measurement precision below 5%, making results highly reliable for downstream analysis. Importantly , the recommended protocol is simplified to be deployable to any cell culture lab without the need for specialized equipment or expertise. This novel workflow leverages the standard Proteograph XT Assay Kit and SP100 automation instrument extending the Proteograph application menu and unlocking powerful capabilities for cellular proteome analysis.

Study Design

Sample information

HeLa and HEK293 cell line pellets were procured from a third-party supplier (HD Biosciences, San Diego). Briefly, HeLa and HEK293 cell lines were grown to appropriate confluence in cell culture flasks, harvested through trypsinization, and centrifuged to pellet before frozen storage. All samples were received and stored at –80 °C before sample processing.

Sample preparation

Cell line pellets were resuspended in the following lysis buffers to two different starting concentrations of 1×10^6 cells/mL and 4×10^6 cells/mL:

- **XT Lysis 1 buffer**: 25 mM Tris (pH 7.2), 0.5% Triton X-100, 100 mM KCl, 10% glycerol
- **XT Lysis 2 buffer**: proprietary formulation
- **IP Lysis buffer**: 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol
- **RIPA buffer**: 25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS

All cell lysis buffers were supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (100X), EDTA-Free (Thermo Fisher Scientific, Cat. No. 87785) before cell lysis. Cell pellets underwent either vortex or Dounce homogenizer-based lysis. For vortex lysis, samples were resuspended in XT Lysis 1, IP Lysis, or RIPA buffers and were incubated for 15 min on ice with vortex for 10 sec every 5 minutes. Cell lysates were centrifuged at 14,000*g* for 15 minutes at 4 °C. Samples in XT Lysis 2 buffer were vortex lysed with a modification of vortex for 30 sec every 5 minutes and cell lysates were centrifuged at 700*g* for 10 min at 4 °C. For Dounce lysis, samples were resuspended in XT Lysis 2 buffer

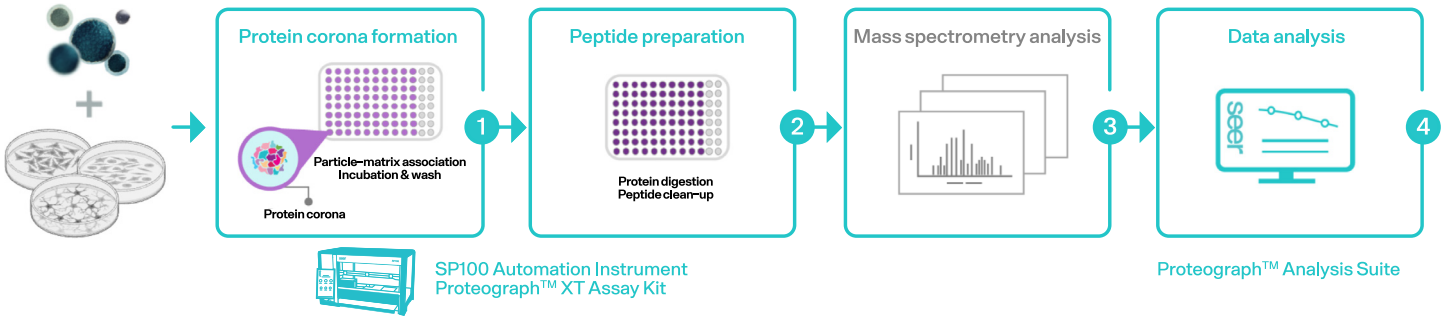


Figure 1. Proteograph XT workflow. **(1)** Cell lysate samples are added to Seer's NP suspensions and a protein corona is formed. **(2)** Proteins are digested and the resulting peptide mixture is desalted and quantified. **(3)** Peptides are injected onto an LC-MS system. **(4)** LC-MS raw data are transferred to the PAS for peptide and protein identification.

and homogenization was performed by 60 passes of the pestle. Cell lysates were centrifuged at 700*g* for 10 min at 4 °C. In all cases, clarified cell lysate supernatant was collected after centrifugation, subjected to BCA protein quantification, and stored at –80 °C until Proteograph processing. For each sample, 240 µL of volume was transferred to a Seer sample tube and the standard Proteograph XT assay protocol was performed (n=3).

Sample preparation using the Proteograph XT is shown in Figure 1 with the following steps. (1) Upon addition of biologic samples to Seer's NP suspensions, a stable and reproducible protein corona is formed based on the particle physicochemical properties. Protein corona-containing NPs are captured and washed, taking advantage of the paramagnetic core. (2) Proteins are then denatured, reduced, alkylated, and digested directly on the particles using an automated one-pot sample preparation workflow, resulting in tryptic peptides released into the supernatant. The resulting peptide mixture is then desalted using solid phase extraction on the SP100 Automation Instrument. Peptides are then quantified using a fluorescence spectrometer, dried, and resuspended on the SP100 Automation Instrument before injection onto a (3) LC-MS system. LC-MS raw data were transferred directly to the Proteograph™ Analysis Suite (PAS) for peptide and protein identification, quantification, and other biological insights. For direct digestion (n=2), sample volume was determined based on protein concentration of each cell lysate and constant mass of ~10 ug was added to the SP100 Automation Instrument without nanoparticles beginning at the protein denaturation step prior to enzymatic digestion and processed in parallel with standard Proteograph XT assay sample processing.

LC-MS data acquisition

For Data-Independent Acquisition (DIA), 8 µL of reconstituted peptide mixture from each NP preparation was analyzed resulting in a constant 400 ng load on column for NP A and NP B samples. Each sample was analyzed with a Vanquish NEO nanoLC system coupled with a Orbitrap™ Astral™ (Thermo Fisher, Germany) mass spectrometer using a trap-and-elute configuration. First, the peptides were loaded onto an Acclaim™ PepMap™ 100 C18 (0.3 mm ID x 5 mm) trap column and then separated on a Gen1 50 cm µPAC™ analytical column (Thermo Fisher Scientific) at a flow rate of 1 µL/min using an effective gradient of 4 – 35% mobile phase B (0.1% FA, 100 % ACN) mixed into mobile phase A (0.1% FA, 100% water) over 30 min, resulting in 36 min total run time. The mass spectrometer was operated in DIA mode. MS1 scans were performed in the Orbitrap detector at 240,000 R every 0.6 seconds with a 5 ms ion injection time or 500% AGC (500,000 ion) target. For the DIA experiment, two-hundred fixed DIA windows from 380-980 m/z were collected at the Astral detector per cycle with 3 Th precursor isolation windows, 25% normalized collision energy, and 5 ms ion injection times with a 500% (50,000 ion) active gain control maximum. MS2 scans were collected from 150-2000 m/z. Window placement optimization was turned on. A source voltage of 1500 V and an ion transfer tube temperature of 300 °C were used for all experiments.

Data analysis

DIA data was processed using Proteograph™ Analysis Suite. Raw MS data was processed using the DIA-NN search engine (version 1.8.1) in library-free mode searching MS/MS spectra against an in silico generated spectral library of human protein entries (UP000005640_9606). Library-free search parameters include trypsin protease, 1 missed cleavage, N-terminal Met excision, fixed modification of Cys carbamidomethylation, no Met oxidation, peptide length of 7-30 amino acids, precursor range of 300-1800 m/z, and fragment ion range of 200-1800 m/z. MS1 and MS2 mass accuracy was set to 3 and 8 ppm, respectively. Precursor and Protein Group FDR thresholds were set at 1%. Quantification was performed on summed abundances of all unique peptides considering only precursors passing the FDR thresholds. PAS summarizes all nanoparticle values for a single protein into a single quantitative value. Specifically, a single protein may have been measured up to two times, once for each nanoparticle suspension. To derive the single measurement value, PAS uses a maximum representation approach, whereby the single quantification value for a particular peptide or protein group represents the quantitation value of the NP most frequently measured across all samples.

Results

Proteograph XT nanoparticle technology enables deep cellular proteome analysis capable of measuring over 11,000 proteins.

To understand the efficacy of the Proteograph XT platform for cellular proteome analysis, we evaluated the impact to overall proteome coverage when different cell lysis buffers, different lysis techniques, and variable amounts of cell pellet are utilized. A collection of cell lysis buffers was selected from those commonly used for protein assay experiments including RIPA, IP lysis, as well as in-house formulations, including Tris-Triton X-100 (XT Lysis 1) and XT Lysis 2 (proprietary formulation). Buffers formulated for non-denaturing, low detergent conditions (IP lysis) and enhanced solubilization of cell matrices (XT Lysis 1 + 2) were prioritized to preserve

native protein structure and provide favorable conditions for nanoparticle:protein binding. Additionally, we investigated the use of Dounce homogenization and whether shear stress offered more complete cell lysis and deeper proteome coverage when combined with the Proteograph XT workflow (Dounce XT Lysis 2). For benchmarking our results, we included i) RIPA buffer representing a popular choice for lysis and protein extraction of mammalian cells, and ii) direct digest preparations to understand the baseline proteome coverage when nanoparticles are not included in each lysis buffer preparation.

The observed cellular proteome depth achieved in HeLa and HEK293 with Proteograph XT surpassed the measurement coverage of conventional direct digest (DD) conditions, with approximately 10% more protein IDs (Figure 2). Performance of XT Lysis 1, XT Lysis 2, Dounce XT Lysis 2, and IP Lysis buffers were relatively similar across both cell lines and starting cell concentrations, however, XT Lysis 1 was most consistently the top performing cell lysis buffer. With RIPA buffer, Proteograph XT coverage was less than the DD control, likely due to denaturation effects or its formulation with two ionic and one non-ionic detergents that interfere with nanoparticle:protein binding. The difference in protein IDs was slightly less at 1 × 10^6 cells/mL compared to 4 × 10^6 cells/mL with <10% difference for all tested conditions, indicating flexibility of the workflow to accommodate cell culture growth constraints. Collectively, these results suggest 4 × 10^6 cells/mL with XT Lysis 1 and vortex lysis as the recommended approach for cellular proteome analysis with Proteograph XT assay.

High-value proteins across diverse cellular compartments, functions, and structures are captured more extensively with Proteograph XT

To more systematically evaluate the composition of proteins detected, and whether the Proteograph XT nanoparticles are preferentially enriching or stabilizing different subsets of the proteome, we performed a UniProtKB keyword[1] enrichment analysis. Keywords were included for the categories of 'Cellular component', 'Domain', 'Molecular function' and 'Post-translational modification' to examine a variety of functional and structural protein classes. Comparing unique proteins detected in RIPA DD as a baseline reference, 13 keyword categories were identified where Proteograph XT was able to consistently capture and identify significantly more unique proteins, in both HeLa and HEK293 samples (Figure 3A). Notably, a substantial percent increase in unique 'Nucleus' proteins (103-110%) and nucleus-related 'DNA-binding' (189–256%) and 'Zinc finger' (314-396%) proteins was observed in the Proteograph XT compared to DD (Figure 3B). Similarly, improvements in unique 'Cell membrane' proteins (34–71%) and surface membrane-

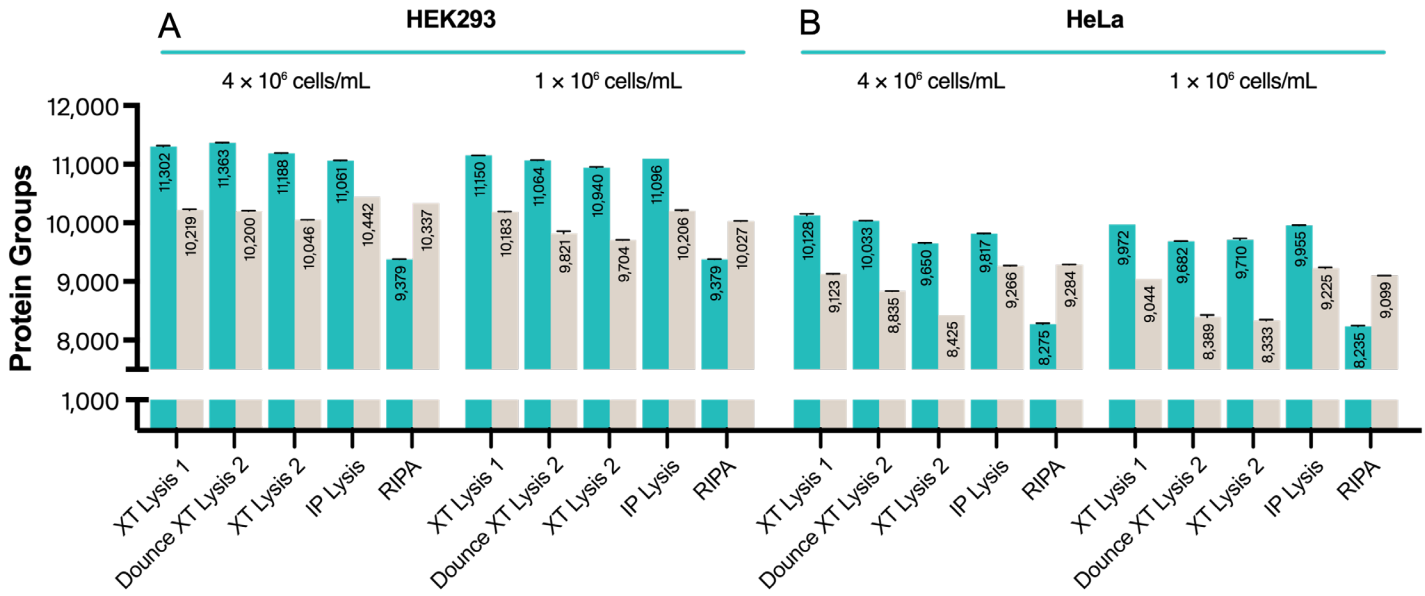


Figure 2. Protein identifications for different cell lysate conditions using the standard Proteograph XT workflow (teal) and comparison to direct digestion workflow (grey) for HEK293 **(A)** and HeLa **(B)** preparations. Mean protein identification numbers shown with error bars denoting standard error of the mean (n= 3).

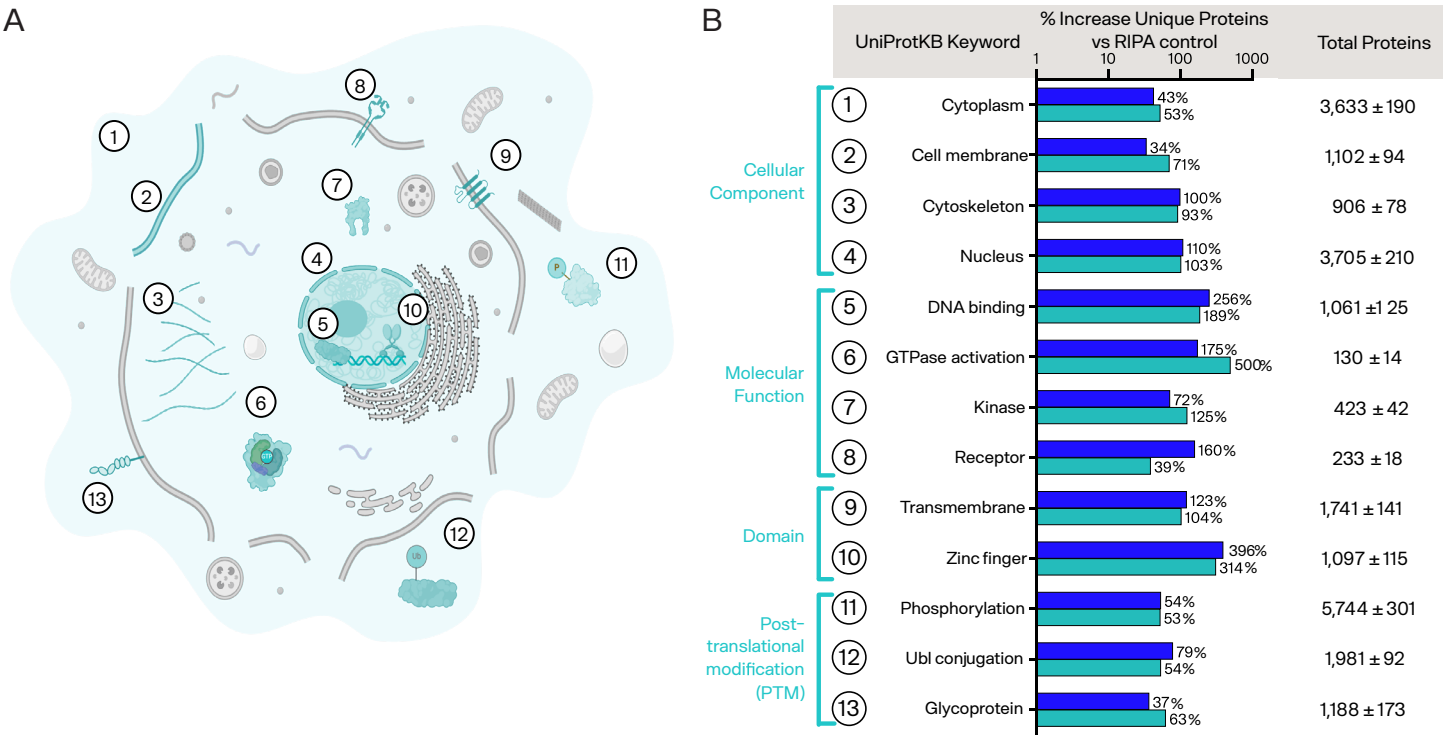


Figure 3. **(A)** Cellular schematic depicting 13 UniProtKB keywords where the proportion of uniquely identified proteins was significantly enhanced by Proteograph XT. **(B)** The percent increase in uniquely identified proteins for Proteograph XT compared to RIPA DD control is shown for HeLa and HEK293 conditions from XT Lysis Buffer 1. Data represents values from 4 × 10^6 cells/mL results. The overall Total Proteins count represents the average number (± standard deviation) of proteins identified for each keyword category in HeLa (n=3) and HEK293 (n=3) experiments.

related 'Receptor' (39-160%), 'Transmembrane' (104-123%), and 'Phosphorylation' (53-54%) proteins were observed—of specific interest to drug discovery and surfaceome research. Enhanced measurement of high-value and difficult to characterize protein compartments like the surface membrane and nucleus, as well as proteins known to have higher order molecular structures and PTMs, can now be studied more extensively with the Proteograph XT cell lysis protocol.

Proteograph XT probes the full range of cellular proteome abundance, from tens to millions of protein copies per cell

Achieving comprehensive proteome analysis represents a significant challenge in the field of proteomics. This level of analysis is crucial for advancing systems biology research, understanding drug perturbations and identifying potential biomarkers. However, despite substantial advancements in proteomics technologies over the past decades, fully characterizing the proteome of human cells has proven difficult, primarily due to the vast dynamic range—spanning at least seven orders of magnitude—present within the cellular proteome. As a rough proxy for the sensitivity and protein abundance depth achieved with the Proteograph XT cell lysis workflow, we mapped our results against a HeLa reference proteome[2] – one of the deepest of a human single-cell type to date. The establishment of protein copy numbers for ~14,000 protein isoforms in their study, at an MS analysis rate of just over 1 sample/day, served as a useful measuring stick for understanding the comprehensiveness of the Proteograph XT approach, cataloging 10,000-11,000 protein groups at an MS analysis rate of just over 1 sample/hour. For our HeLa dataset, 9,986 total protein groups were found consistently in all replicates, and 9,121 were matched to copy numbers in the reference dataset, spanning an impressive 6.5 orders of magnitude (Figure 4A). Remarkably, compared to DD control the Proteograph XT results demonstrated enhanced access to ultra-low copy number targets, with 41% more coverage of proteins estimated to have less than 500 copies/cell (Figure 4B). The Proteograph XT workflow was the only method capable of detecting DENND2C, a guanine nucleotide exchange factor (GEF), the lowest reported protein in the database at an estimated 14.33 copies per cell.

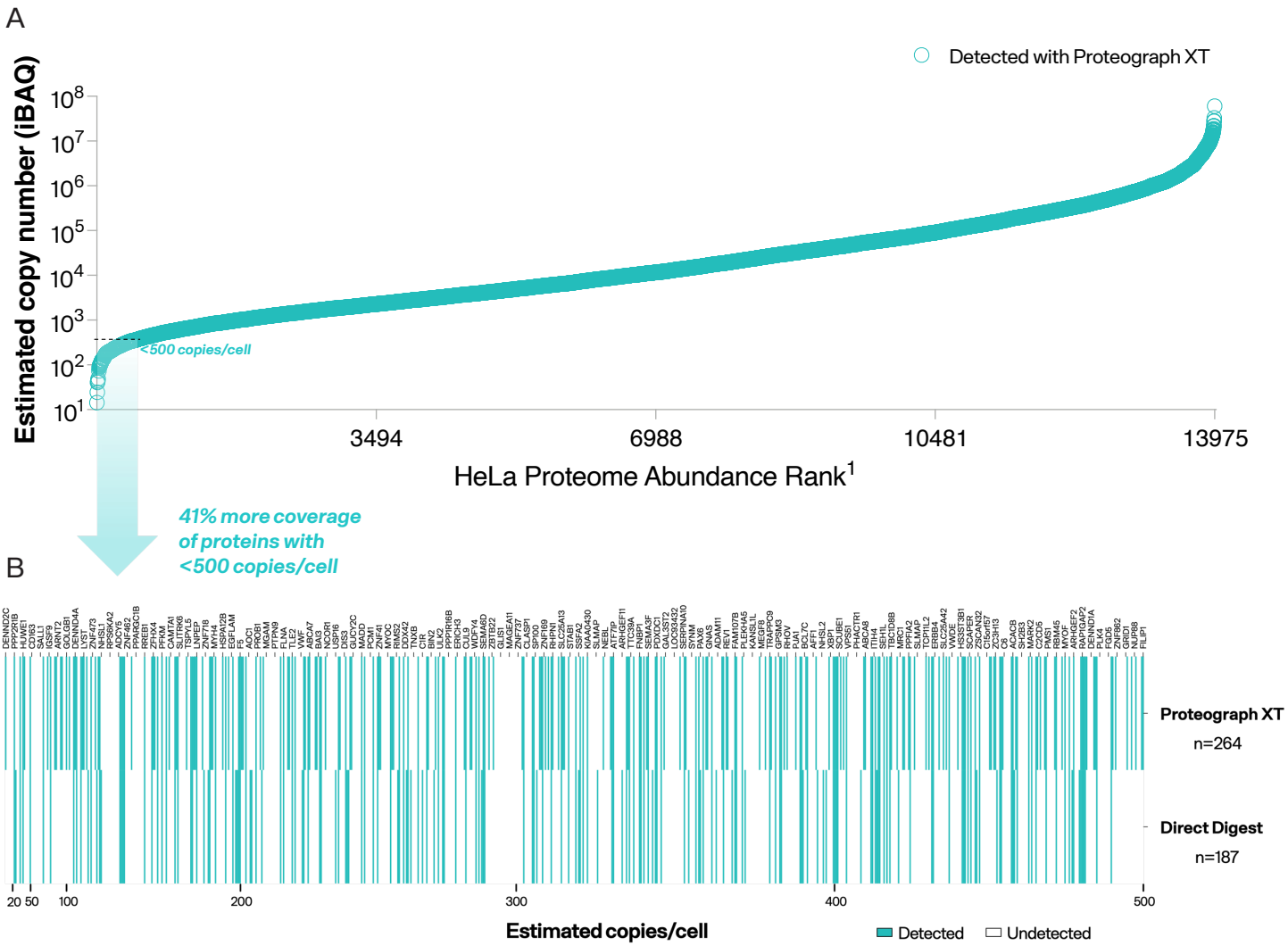


Figure 4. **(A)** In HeLa, from detected 10,251 protein groups a total of 9,121 protein groups were matched to a HeLa reference database from Bekker-Jensen et al., (2017), spanning a copy number range of 6.5 orders of magnitude. Quartile index protein number shown on x-axis. **(B)** For proteins estimated to be expressed at less 500 copies per cell, Proteograph XT detected 264 proteins compared to 187 in a conventional RIPA cell lysate workflow. The protein concentrations are rank ordered in increasing abundance from left to right, every 6th protein labeled above heatmap to improve legibility. (Proteins mapped from HeLa XT Lysis Buffer 1 or RIPA DD, 4 × 10^6 cells/mL results).

Proteograph XT standardized, automated cell lysis workflow ensures high measurement reproducibility for highly reliable downstream analysis

Importantly, the quantitative consistency of the Proteograph XT workflow was maintained even with the increased depth of coverage achieved. In triplicate analysis of HeLa and HEK293 cell lines using the XT Lysis 1 protocol, measurement precision was high with median protein group intensity %CV of less than 5% across the entire measured proteome (Figure 5A). Of equal importance, measurement precision was not distorted for low abundant copy numbers as triplicate %CVs for proteins estimated to be less than or equal to 500 copies/cell were 6.2% and 5.9% for HeLa and HEK293, respectively (Figure 5B). These results collectively demonstrate the robustness and reliability of Proteograph XT to conduct quantitative cellular proteomic analysis, and a trusted platform for the elucidation of biological distinctions in cellular proteomes across varying experimental conditions.

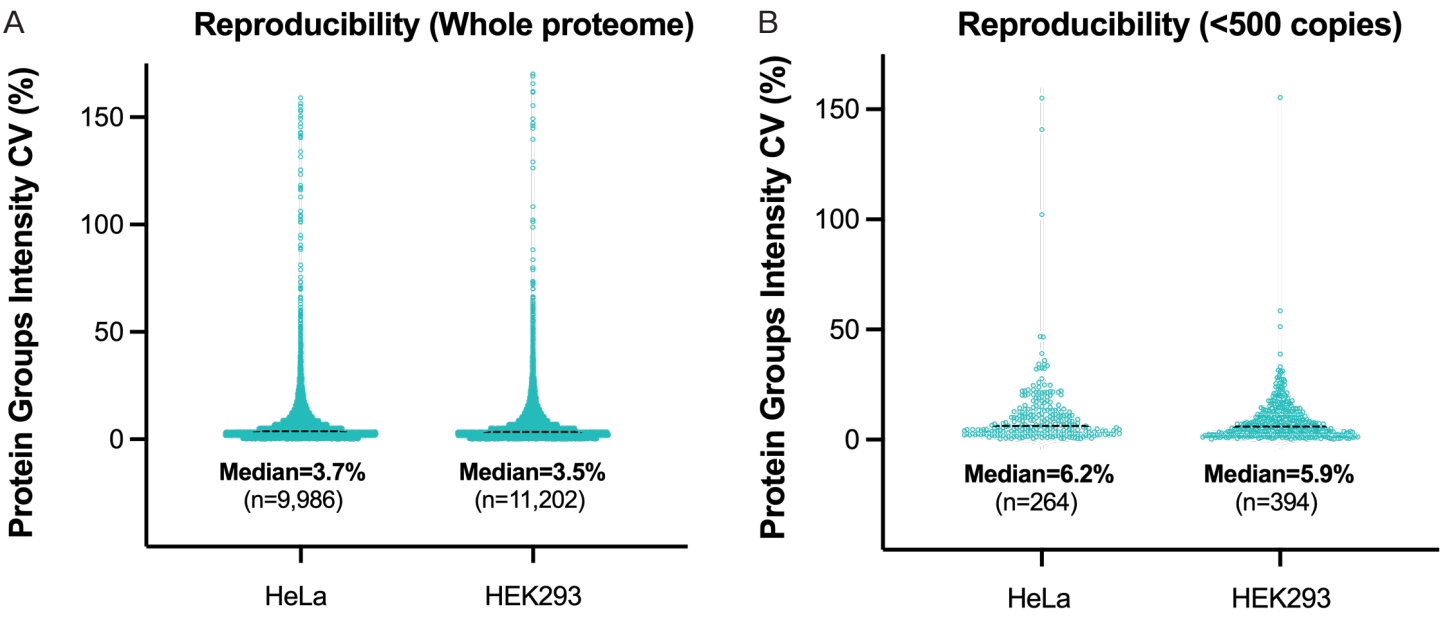


Figure 5. High quantitative consistency (median CV < 5%) across the entire measured proteome **(A)**, even for low-abundance proteins **(B)**, further underscores the robustness and reliability of the platform. Protein Group intensity CV (%) values represent HeLa XT Lysis Buffer 1, 4 × 10^6 cells/mL results (n = 3), with dotted lines denoting median CV.

Summary

This study demonstrates the significant advantages of using the Proteograph XT platform with a simplified cell lysis protocol for comprehensive and reproducible cellular proteomics applications. By combining a streamlined cell lysis procedure with engineered nanoparticle technology and automated sample preparation, Proteograph XT overcomes key limitations of traditional MS-based proteomics, including challenges associated with sample preparation variability, detection of low-abundance proteins, and efficient extraction of proteins from diverse subcellular compartments. The recommended workflow achieves significantly enhanced proteome coverage, routinely identifying 10,000–11,000 proteins in HeLa and HEK293 cell lines, representing a 10% increase in protein IDs and a >40% improvement in detecting low-abundance proteins (<500 copies/cell) compared to conventional methods. Notably, Proteograph XT demonstrates superior capture of critical protein classes, including nuclear, cell membrane, and DNA-binding proteins, crucial for understanding cellular signaling and disease pathways. High quantitative consistency (CV < 5%) across the measured proteome, even for low-abundance proteins, further underscores the robustness and reliability of the platform. This simplified, automated workflow, deployable in any cell culture lab, empowers researchers to unlock unprecedented depth in intracellular proteomics, facilitating more comprehensive and reliable biological insights.

References

1. Nucleic Acids Research, 6 January 2025, Pages D609–D617, https://doi.org/10.1093/nar/gkae1010; (https://www.uniprot.org/keywords?query=*)
2. Cell Syst. 2017 Jun 28;4(6):587–599.e4. doi: 10.1016/j.cels.2017.05.009

Appendix: Proteograph™ XT Protocol for Cellular Proteome Analysis

This protocol outlines the steps for efficient protein extraction from cultured cells, ensuring favorable conditions for nanoparticle-based enrichment with the Proteograph XT Assay.

Note: The following protocol has been empirically developed to maximize native protein conditions using non-denaturing components and minimal detergents to ensure Proteograph XT Assay and downstream LC-MS compatibility. For alternative lysis buffer options please consult with your Seer Field Application Scientist representative.

Generic Cell Harvest Procedure

As shown in Figure 6, harvest the cells using an appropriate dissociation reagent (e.g., Trypsin-EDTA) according to the standard procedures in your lab. Wash the cell pellet with PBS to remove any residual culture medium, contaminants, and dissociation reagent. At this stage, perform a cell count, then centrifuge the remaining sample to collect the cell pellet. Finally, store the cell pellet at -80 °C until ready for cell lysis or proceed directly with the lysis procedure.

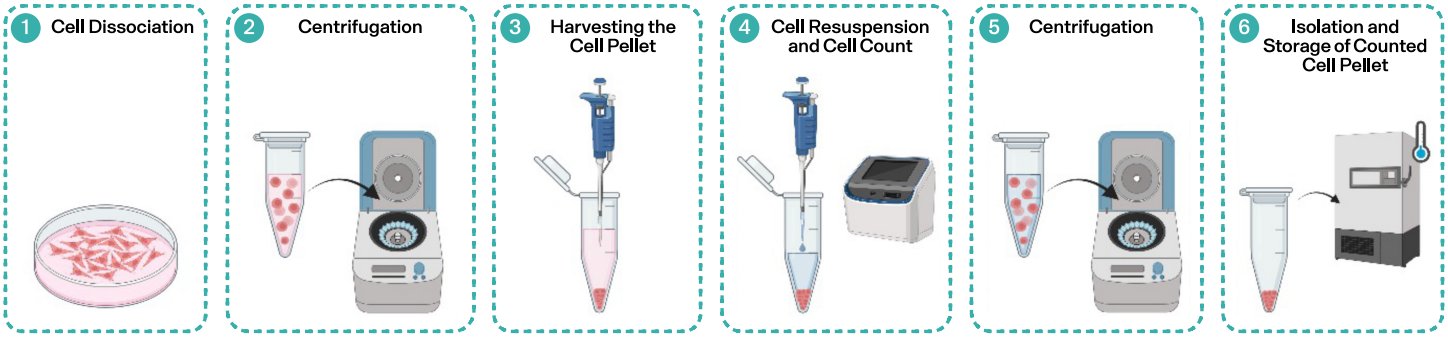


Figure 6. Cell harvest procedure

Cell Lysis Procedure

This protocol is designed to obtain a cell lysate with protein extraction from 4 × 10^6 cells/mL using XT Lysis Buffer 1 and vortex based cell lysis. Note that a different cell concentration may affect extraction efficiency and Proteograph XT results (Figure 7).

XT Lysis Buffer 1

- 25 mM Tris (pH 7.2)
- 0.5% v/v Triton X-100
- 100 mM KCl
- 10% v/v glycerol

To prepare 50 mL of XT Lysis buffer 1, mix the following:

- 25 mM Tris (pH 7.2): 44.75 mL
- Triton X-100: 250 µL
- KCl: 373 mg
- Glycerol: 5 mL

Protocol

1. Thaw out the cell pellet on ice.
   - Aliquot enough volume of XT Lysis buffer 1 and add 1/100 volume of Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X) (Thermo Fisher Scientific, Cat. No. 87785) to the buffer.
   - Add the ice-cold lysis buffer (supplemented with protease inhibitors) to the cell pellet to achieve a final cell concentration of 4 × 10^6 cells/mL. Resuspending 2 × 10^6 cells in 500 µL of lysis buffer is recommended, in order to obtain adequate cell lysate volume for one Proteograph XT Assay replicate.
2. Incubate the cell suspension on ice for 15 minutes. Vortex briefly (10 seconds) every 5 minutes.
3. Centrifuge the lysate at 14,000 *g* for 15 minutes at 4 °C.

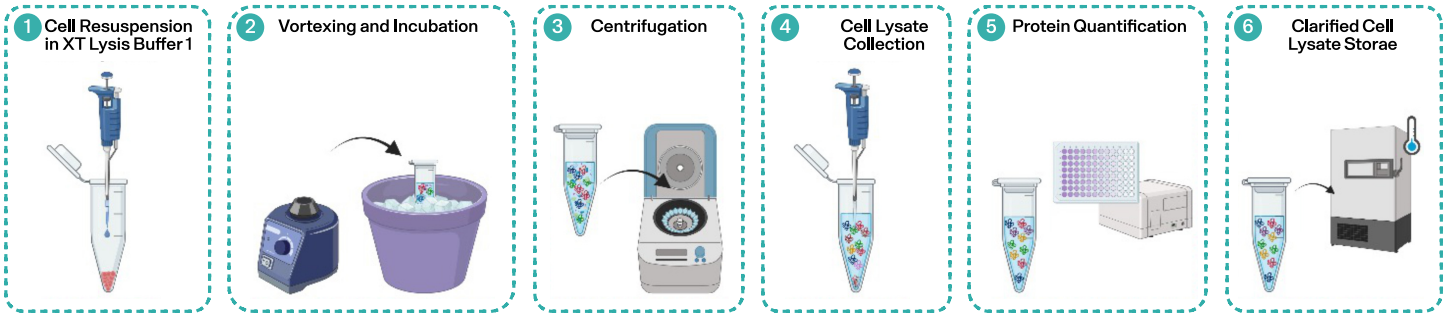


Figure 7. Cell lysis workflow

4. Carefully transfer the supernatant to a new vial, avoiding the pellet.
5. Use a BCA protein assay kit to determine the protein concentration of the lysate.
6. Store the cell lysate at -80 °C until you are ready to proceed with the Proteograph XT Assay.

Proteograph XT workflow

Cell lysate samples can be processed on Proteograph utilizing the standard Proteograph XT Assay workflow and SP100 Automation Instrument.

Notes

- The starting volume of the lysis buffer can be adjusted based on the cell count to reach a final concentration of 4×10^6 cells/mL. Ensure that at least 300 µL of lysate is obtained after centrifugation to proceed with the Proteograph XT assay.
- Lower cell concentrations may reduce the depth of proteome coverage. Seer has data available for cell concentration of 1×10^6 cells/mL for reference.
- Maintaining consistent cell concentrations across study samples is *crucial* for relative quantification of proteins identified in the Proteograph XT assay. The user must perform cell counting after harvesting cells from culture dishes to ensure consistency across study samples.

Find out more at
seer.bio/product/proteograph-product-suite