illumina®

Illumina Protein Prep

NGS-based solution for deeper insights into the proteome

Measure 9.5K unique human proteins in a single plasma or serum sample using the SomaScan® proteomics assay with NGS readout

Go from sample to results in < 2.5 days with just 4 hours hands-on time following a streamlined, automated workflow

Analyze proteomic data with integrated secondary analysis via DRAGEN™ Protein Quantification and Illumina Connected Multiomics

Introduction

Proteins play a key functional role in human biology, reflecting a real-time snapshot into health and disease states. Insights from proteomics serve as a critical link between genotype and phenotype, enable a deeper understanding of disease mechanisms, and, ultimately, help with the prediction, monitoring, and prevention of disease escalation. High-throughput proteomics assays with next-generation sequencing (NGS)–based readouts enable large-scale proteomic studies and have the ability to connect genomic and proteomic data sets, accelerating multiomics research.

Illumina Protein Prep is a comprehensive high-throughput proteomics solution that integrates trusted Illumina sequencing by synthesis (SBS) chemistry with the high sensitivity of the Standard BioTools SomaScan proteomics assay. This innovative proteomics assay uses SOMAmer® (slow off-rate modified aptamer) Reagents for protein capture to achieve high specificity for target proteins compared to antibody-based approaches.[1] Combining this advanced proteomics assay with NGS-based readout and the bioinformatics power of Illumina data analysis software gives researchers a streamlined sample-to-insights solution for evaluating 9.5K unique human proteins in a single plasma or serum sample (Figure 1).

Comprehensive content

The Illumina Protein Prep assay enables discovery and quantification of 9.5K unique human proteins in plasma or serum samples using more than 10,000 SOMAmer Reagents. This comprehensive content* targets human proteins across a diverse set of pathophysiological processes, including cancer, inflammation, immunology, and cardiometabolic function. The content covers major molecular targets, including receptors, kinases, growth factors, and hormones, spanning secreted, intracellular, and extracellular proteins across more than 200 biological pathways.

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* Complete menu of proteins available upon request.

Figure 1: Overview of the Illumina Protein Prep solution

Prepare libraries

Automated SOMAmer-based proteomics assay and Illumina library prep

Sequence

Scalable sequencing with the NovaSeq™ X Series and NovaSeq 6000 System

Analyze data

Integrated secondary data analysis with the DRAGEN Protein Quantification pipeline

Generate insights

Visualization and combined modality studies on Illumina Connected Multiomics[a]

The high-throughput proteomics solution uses the SomaScan proteomics assay for high-plex protein detection from serum or plasma samples, followed by Illumina library preparation and sequencing on the NovaSeq 6000 or NovaSeq X System. Data analysis is performed with integrated secondary analysis via DRAGEN Protein Quantification and Illumina Connected Analytics followed by optional tertiary analysis with Illumina Connected Multiomics for deeper biological insights. The proteomics assay and library preparation workflow is automated on the Illumina Protein Prep Automation System, a custom Tecan Fluent 780, to deliver consistent and reproducible results.

a. Illumina Connected Multiomics will be available in the second half of 2025.

All SOMAmer Reagents used in Illumina Protein Prep have undergone rigorous characterization and the specificity of the SOMAmer Reagent for its cognate protein has been confirmed by orthogonal methods, such as mass spectroscopy and enzyme-linked immunosorbent assay (ELISA).[1] Over 7400 SOMAmer Reagents have at least one additional form of orthogonal validation. With the content included in the Illumina Protein Prep solution, researchers can access deep insights into the proteomics landscape, enabling the discovery of novel biomarkers, drug targets, and insights into disease mechanisms.

Sensitive and precise protein detection

Illumina Protein Prep uses innovative slow off-rate modified aptamers, or SOMAmer Reagents, as protein affinity molecules to achieve exceptional sensitivity and specificity for protein detection across a broad dynamic range.[2] These aptamers, which are short pieces of single-stranded DNA with hydrophobic modifications, provide a high degree of shape matching to the protein target, allowing discernment between nearly identical proteins. Unlike polyclonal antibodies, which can be variable in structure and performance, SOMAmer Reagents are based on binding kinetics, providing femtomolar sensitivity[2] and excellent reproducibility with a low median coefficient of variance (CV) of ~5.5%[†] (Table 1).

Table 1: Summary of expected performance metrics[a]

Sample	Median intra-run CV	Median inter-run CV	Median total-run CV	90th %ile total-run CV
Plasma	4.7%	5.3%	5.8%	8.7%
Serum	4.7%	5.0%	5.0%	7.4%

a. Expected median CV calculated using samples from healthy donors.

Hybridization-based NGS assay

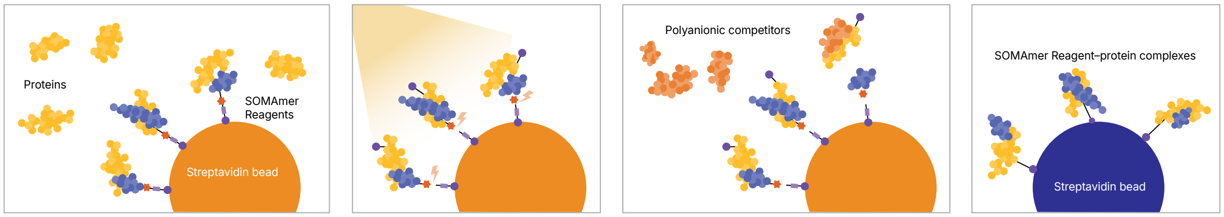
The first step in the Illumina Protein Prep workflow is the SomaScan assay, which quantitatively transforms the protein epitope availability in a biological sample into a specific SOMAmer Reagent–based DNA signal.[3] The initial SOMAmer Reagent–protein binding step is followed by a series of bead capture and wash steps to convert relative protein concentrations into SOMAmer Reagent abundancies (Figure 2). Next, SOMAmer Reagents are converted into barcoded sequencing libraries using a hybridization-based approach (Figure 3). Pairs of probes are hybridized to SOMAmer Reagents in an overnight incubation step before being captured on magnetic beads. Each SOMAmer Reagent has a unique pair of probes, one of which carries a barcode corresponding to a particular SOMAmer Reagent. Unbound probes are washed away to ensure the relative abundance of the SOMAmer Reagents is converted to the abundance of the barcoded probe. Index PCR primers are added to append sample indexes for sequencing and amplified to create individually indexed, barcoded libraries. The entire Illumina Protein Prep assay is automated on a single platform, the Illumina Protein Prep Automation System. Samples are pooled and sequenced on the NovaSeq™ 6000 or NovaSeq X System.

Scalable, streamlined workflow

The Illumina Protein Prep solution uses a streamlined end-to-end workflow (Figure 1) that starts with human plasma or serum samples, followed by highly sensitive protein capture using innovative SOMAmer Reagents and Illumina library preparation. The entire workflow is automated on the Illumina Protein Prep Automation System, enabling labs to go from samples to results in less than 2.5 days with ~4 hours of hands-on time (Table 2). Libraries are sequenced on the NovaSeq 6000 System (S4 flow cell) or the NovaSeq X System (10B flow cell), with 170 samples and 22 controls per run. The NovaSeq X Series 25B flow cell accommodates 340 samples and 44 controls per run.

[†] Expected median CV calculated using samples from healthy donors.

Figure 2: Protein-capture using the SOMAScan assay



Protein capture using SOMAmer Reagents (blue)

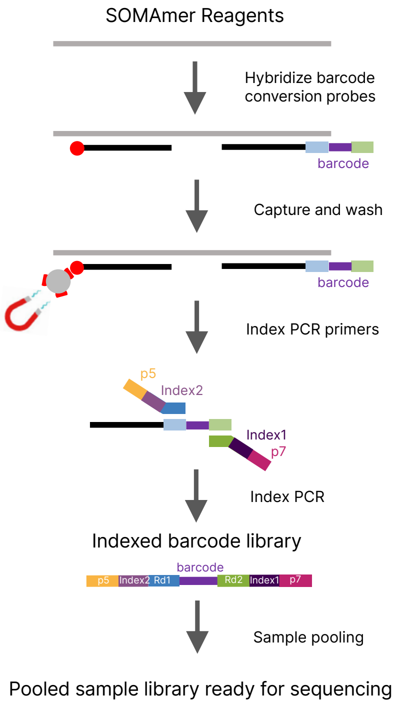
UV light releases complexes back into solution

Specific complexes remain bound. Polyanionic competitor prevents rebinding of nonspecific complexes

Biotinylated SOMAmer Reagent–protein complexes bind to streptavidin beads

SOMAmer Reagents (blue) contain a photocleavable linker and biotin. SOMAmer Reagents bound to streptavidin beads are used to capture specific proteins (yellow) from a complex mixture of proteins in serum or plasma. Unbound proteins are washed away and bound proteins are tagged with biotin. Next, UV light is used to break the photocleavable linker and release SOMAmer–protein complexes back into solution. During the incubation step, nonspecific complexes dissociate while specific complexes remain bound. The inclusion of polyanionic competitors during the incubation step prevents nonspecific rebinding of dissociated proteins. Specific protein-SOMAmer Reagent complexes are captured on new streptavidin beads and are eluted for relative quantification using NGS.

Figure 3: Illumina Protein Prep NGS conversion chemistry



Following the SomaScan assay, SOMAmer Reagent abundanciesare converted to barcoded sequencing-ready libraries using a hybridization-based approach.

After sequencing is complete, the DRAGEN Protein Quantification pipeline is autolaunched, generating normalized protein expression counts and quality control reports. These output files are analyzed easily with tertiary analysis platforms, including Illumina Connected Multiomics, enabling deeper biological insights into the proteome.

Integrated data analysis

Labs can analyze data easily using a fully integrated proteomic secondary data analysis pipeline that includes NGS and proteomic assay–specific normalization methods. Following sequencing, the DRAGEN Protein Quantification pipeline is autolaunched via Illumina Connected Analytics, a secure, streamlined, cloud-based platform to scale up secondary analysis and reduce manual touchpoints. Integration with the Illumina Connected Multiomics platform simplifies data visualization and enables combined modality studies.

Read the Illumina Protein Prep data normalization technical note to learn more.

Summary

The Illumina Protein Prep solution is a comprehensive, end-to-end proteomics workflow with NGS-readout for large-scale protein studies. This high-performance assay leverages innovative SOMAmer Reagents to detect 9.5K unique human proteins from a single plasma or serum sample with femtomolar sensitivity and excellent reproducibility. The comprehensive content of Illumina Protein Prep contains rigorously validated protein-affinity reagents, covering key biological processes, including cancer, inflammation, immunity, cardiometabolic function, and more. By combining this high-plex proteomics assay with an NGS readout, Illumina Protein Prep solution enables the integration of proteomic data with genomic and transcriptomic data, paving the way for high-impact multiomics research.

Table 2: Illumina Protein Prep specifications

Parameter	Specification
Sample type	Plasma or serum
Input volume	55 µl
Total no. of SOMAmer Reagents	> 10,000
No. of human proteins targeted	~9,500 unique human proteins
Dynamic range	> 10-log (fM to mM)
Throughput	Up to 384 reactions per week: • 170 samples + 22 controls per run on NovaSeq 6000 S4 or NovaSeq X 10B flow cells • 340 samples + 44 controls per run on the NovaSeq X 25B flow cell
Total workflow time	2.5 days
Hands-on time	~4 hours

Learn more →

Illumina Protein Prep

NGS-based proteomics

NovaSeq 6000 System

NovaSeq X Series

DRAGEN secondary analysis

Illumina Connected Analytics

References

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2. SomaLogic. SomaScan 7K Assay v4.1 technical note. somalogic.com/wp-content/uploads/2023/01/Rev4_2022-01_SomaScan_Assay_v4.11.pdf. Updated January 2022. Accessed January 2, 2024.
3. Gold L, Ayers D, Bertino J, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One*. 2010;5(12):e15004. doi:10.1371/journal.pone.0015004.

Ordering information

Product	Catalog no.
Illumina Protein Prep 9.5K Plasma (96 samples)	20137827
Illumina Protein Prep 9.5K Serum (96 samples)	20137828
Illumina Protein Prep Automation System	20116818

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