QUANTUM SI™

High-confidence Protein Sequencing from Nanogram Inputs with Library Preparation Kit V3

Key Innovations

Unlock scarce samples: Protein libraries are generated from 200 ng or less total protein, delivering high-confidence sequencing from limited protein sources.

Detect low-abundance proteins with confidence: A mixture of five proteins of varying relative concentrations generated high-confidence results down to 1–2 ng (<1% relative abundance).

Identify target proteins from biological specimens: Enhanced bioinformatics assess proteins immunoprecipitated from biological samples, like cell lysates, uniquely differentiating proteins in a mixture with precision.

Fast and familiar workflow: Updated protocol yields sequenceable libraries within 48 hours, with less than two hours of hands-on time.

Ordering information

Product	Catalog number
Platinum® Pro instrument	910-10904-00
Sequencing Kit V4 (4 chips)	910-00038-04
Library Preparation Kit V3 (4 libraries)	910-00012-03

Introduction

Protein research is often limited by how much sample can be obtained for analysis. Many high-value proteomics applications – such as immunoprecipitation studies and low-yield enrichments – fall below the microgram-scale inputs required by traditional workflows, forcing difficult trade-offs or the loss of precious samples.

Quantum-Si's Library Preparation Kit V3 overcomes this constraint by enabling high-confidence, single-molecule protein sequencing from 200 ng or less of total protein on the Platinum Pro instrument. In complex mixtures, proteins with favorable sequence coverage can be confidently detected at 1-2 ng (less than 1% relative abundance) – an important advantage for low-abundance signaling proteins, receptors, and enriched targets.

With a fast, familiar protocol that produces sequenceable libraries in under 48 hours and requires minimal hands-on time, Library Preparation Kit V3 integrates seamlessly into existing research workflows. Together with the Platinum® and Platinum Pro instruments, it provides a powerful solution for researchers seeking confident protein identification from limited, valuable, or highly complex samples.

Reduced Input Requirements for Targeted Protein Research

A major challenge in proteomic analyses is obtaining enough material from scarce or valuable tissues, primary cells, or biological samples. Library Preparation Kit V3 was designed to significantly reduce protein input needed to produce high-quality libraries. The kit calls for 200 ng total protein, 100 times less than required with the previous V2 kit. Libraries were created using the V3 kit from 5 proteins ranging from 6-62 kDa, each at 200 ng, compared to the previous V2 kit that contained 8-40 µg (500 pmol) of each protein. This process achieved comparable peptide coverage with over 95% confidence. (Figure 1A). A mixture of 10 proteins was sequenced using the V3 kit at 200 ng (~20 ng per protein) and the V2 kit with a total protein input ranging from 7.6-33 µg (0.76-3.3 µg per protein), providing improved precision in protein identification with the new V3 kit (Figure 1B).

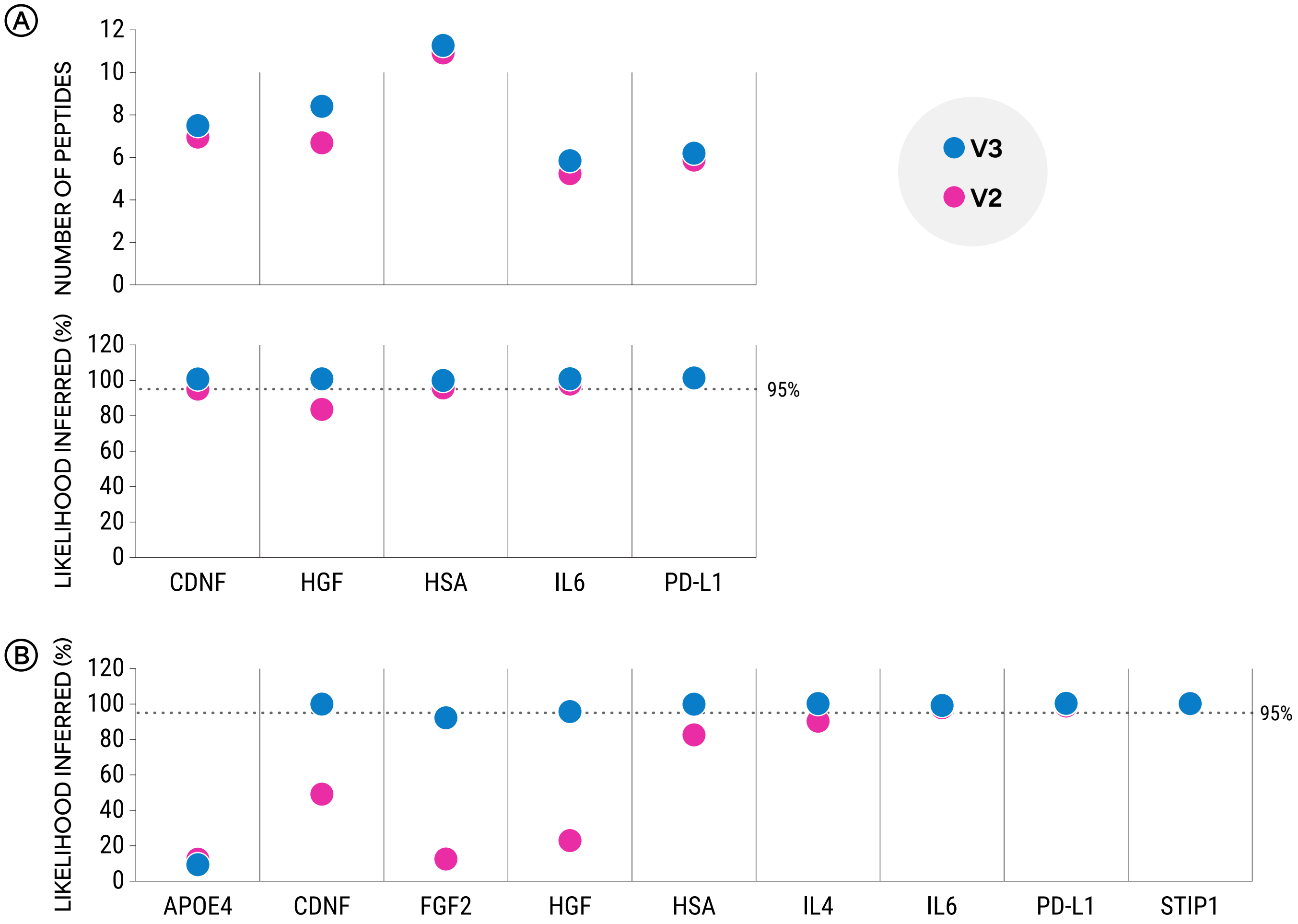

Figure 1. High-confidence protein identification was achieved with Library Preparation Kit V3, as demonstrated on Ⓐ a panel of five recombinant proteins spanning 6-62 kDa, and from Ⓑ equimolar mixtures of ten proteins (200 ng total). Likelihood is the confidence of the presence of a protein in the sample. A likelihood greater than 95% indicates very high confidence.

Detect Low-abundance Proteins with Confidence at the Limit of Detection

Proteins have diverse intrinsic properties (composition, structure, length) that impact their performance across proteomics assays. To characterize the limit of detection of low-abundance proteins in a mixture, a panel of five proteins was titrated in mixtures with a constant total protein of 200 ng per library. Here, ADML was maintained at high abundance (40–196 ng), while four others (LMNB1, PD-l1, HSA, and ILl8R) stepped down from 0.5–40% (1–40 ng) per protein to model performance at low abundance (Figure 2).

Libraries were prepared with Library Preparation Kit V3, sequenced on a full chip using Sequencing Kit V4 on Platinum Pro, and analyzed with the *Protein Inference* v2.17 workflow, referencing the human proteome for alignment.

Highest-coverage proteins (LMNB1, PD-L1, HSA):

- Accurately detected with the highest confidence (≥95% at the lowest abundance)
- LMNB1: reliably inferred at 1 ng in the mixture (0.5% of 200 ng)
- PD-L1 and HSA: reliably inferred at 2 ng (1% of 200 ng)

Lower-coverage proteins (IL18R, ADML):

- Required higher mass fractions for high-confidence detection
- IL18R: required 80 ng (40% of the mixture) to reach high-confidence inference
- ADML: required 160 ng (80% of the mixture) for comparably confident identification

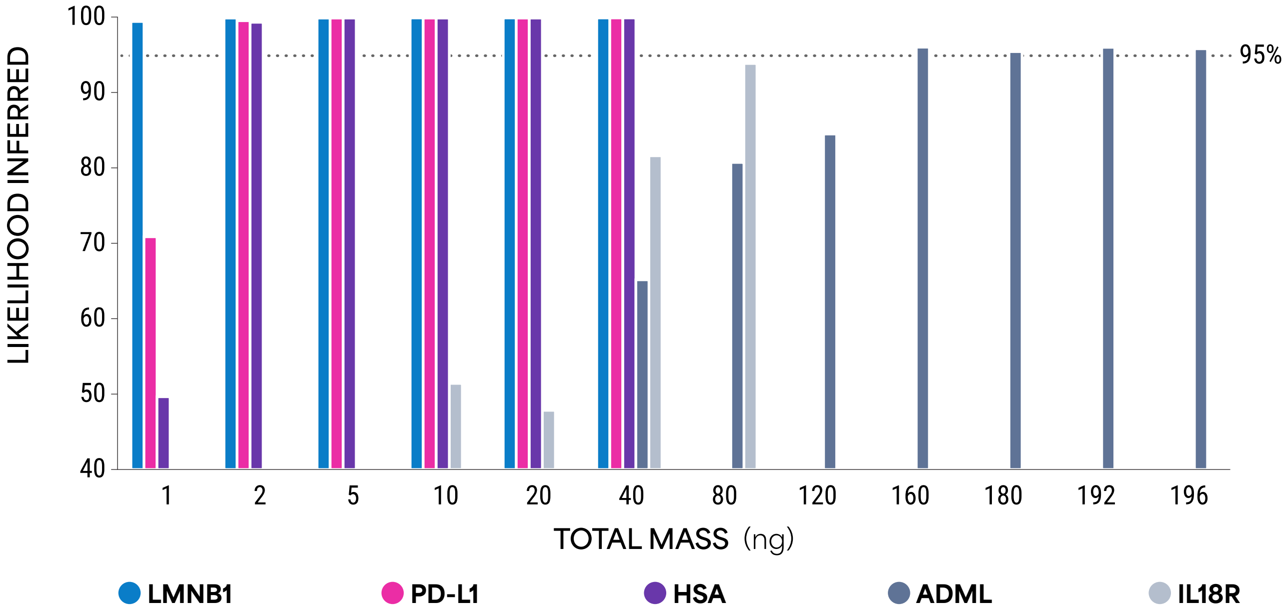

Figure 2. Proteins with the highest coverage (LMNB1, PD-L1, and HSA) can be inferred at 1-2 ng in a 200 ng mixture, <1% relative abundance. Proteins with lower coverage (ADML, IL18R) require higher mass – 80-160 ng – for comparably confident identification.

Accurately Identify Target Proteins from Immunoprecipitated Samples

Targeted proteomics workflows seek to characterize proteins enriched from biological samples like cell lysates, often producing limited mass of total proteins in complex mixtures. Unique identification of proteins in mixtures is enabled by *Platinum Inference* software, which differentiates proteins by their sequence composition and alignment to a reference, even with only nanogram amounts of total protein.

The V3 kit was used to prepare libraries after immunoprecipitating FLAG-tagged integrin alpha-1 (ITGA1) from HEK293 cell extracts, obtained from 10^6 cells in low-nanogram quantities. Libraries were sequenced with Sequencing Kit V4 on Platinum Pro and analyzed with the *Protein Inference* v2.17 workflow. Platinum accurately identified ITGA1 as the protein with top-ranked inference using the whole human proteome as a reference (Figure 3).

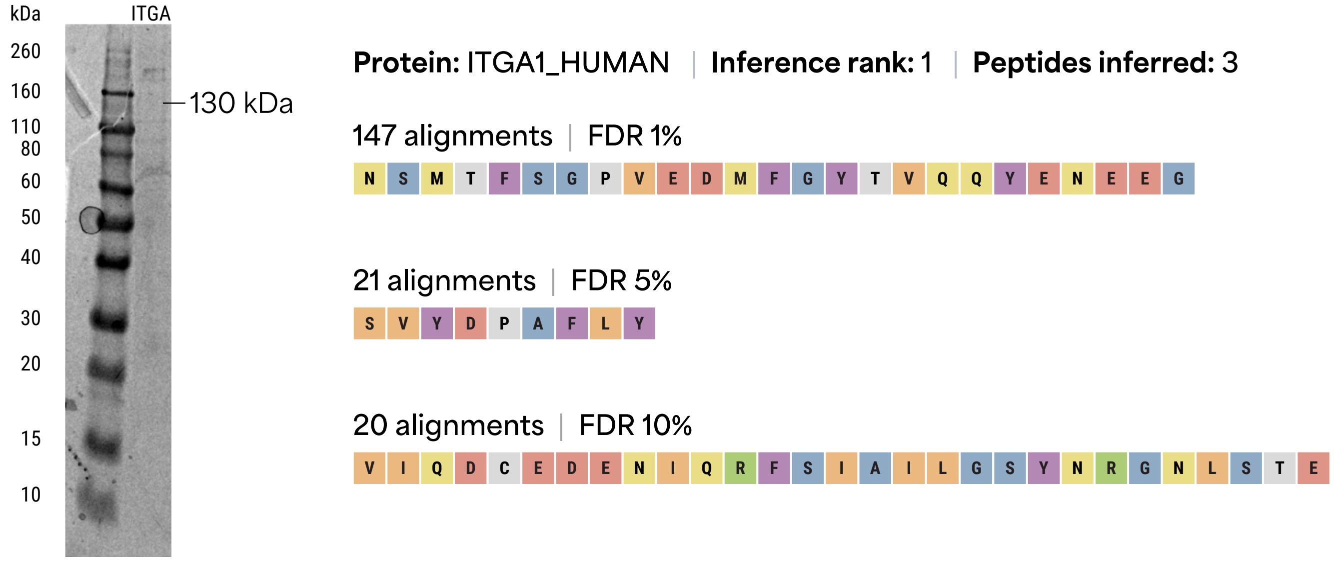

Figure 3. ITGA1 protein, enriched from HEK293 cell extracts, sequenced with Sequencing Kit V4 on Platinum Pro. ITGA1 showed three aligned peptides and was identified with top rank inference.

Conclusions

Library Preparation Kit V3 provides sequence-ready libraries from 200 ng or less of intact proteins or peptides. Combined with improvements to the inference analysis software, it offers high-confidence protein identification on Platinum and Platinum Pro instruments.

When combined with Sequencing Kit V4 and the enhanced *Protein Inference* workflow, protein mixtures of diverse properties can be resolved with confidence. Low-abundance proteins with high coverage can be differentiated down to 1–2 ng, or <1% relative abundance.

Library Preparation Kit V3 is ideal for immunoprecipitation workflows, low-yield enrichments, and protein sequencing from scarce and valuable samples.