Unlock Proteome Depth with the Platinum® Pro Instrument and Sequencing Kit V4

Key Innovations

Next-gen protein sequencing (NGPS): Single-molecule resolution to **identify and characterize proteins** on a benchtop instrument

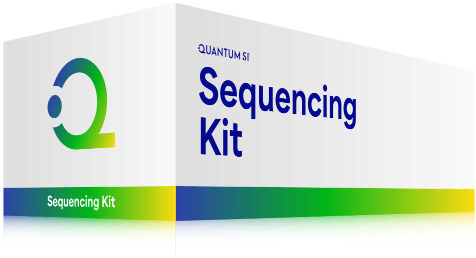
Insights beyond proline: Navigate challenges posed by proline-rich domains, common in **antibodies and membrane proteins**, with Sequencing Kit V4

Expanded coverage: New recognizers, peptidases, and improved bioinformatics offer an **85% increase** in sequenceable peptides

Protein analysis precision: Enhanced bioinformatics unlocks methionine recognition and identifies proteins in mixtures with **greater confidence**

Introduction

Sequencing Kit V4 is for use with the Platinum® and Platinum Pro sequencers. This innovative benchtop platform resolves the amino acid sequences of peptides, based on direct interactions with amino acid recognizers. Upgraded chemistry improves single-molecule sequencing by adding glycine (G) and methionine (M) recognition and enhancing alanine (A) and serine (S) recognition. The inclusion of X-prolyl aminopeptidase allows for cleavage of proline-rich peptides. Improved bioinformatics, including enhancements to amino acid calling and alignment algorithms, delivers higher-quality data with V4, enabling richer data capture on the sequencer.



Sequencing Through Proline

- **V4 detects amino acids after proline**, which makes up 6% of the total amino acids in the human proteome. When the human proteome is digested with Lys-C, over 40% of the resulting peptides contain a proline.
- **Proline is abundant** in vital proteins difficult to analyze by mass spec, common in membrane proteins, antibodies, and transcription factors

Proline's unique structure impacts the peptide fragmentation for mass spec analysis, a phenomenon referred to as the "proline effect," which restricts sequencing by mass spec. The V4 kit delivers improved chemistry that sequences through proline-rich regions, expanding sequencing insights on Platinum (Figure 1).

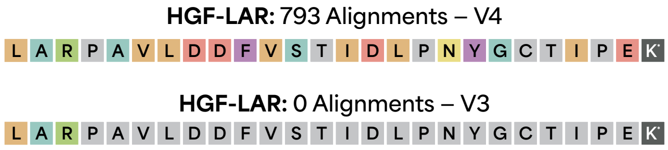


Figure 1. Kinetic signature of the LAR peptide in human growth factor (HGF) reveals three proline residues, now accessible with V4 chemistry.

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Expanded Proteome Coverage

A panel of ten recombinant proteins was digested into peptides and sequenced using the Platinum Pro with both the V4 and V3 kits, demonstrating:

- **Increased peptide alignments** (sequenced copies) in 10 of 10 proteins
- **Increased peptides coverage** in 9 of 10 proteins

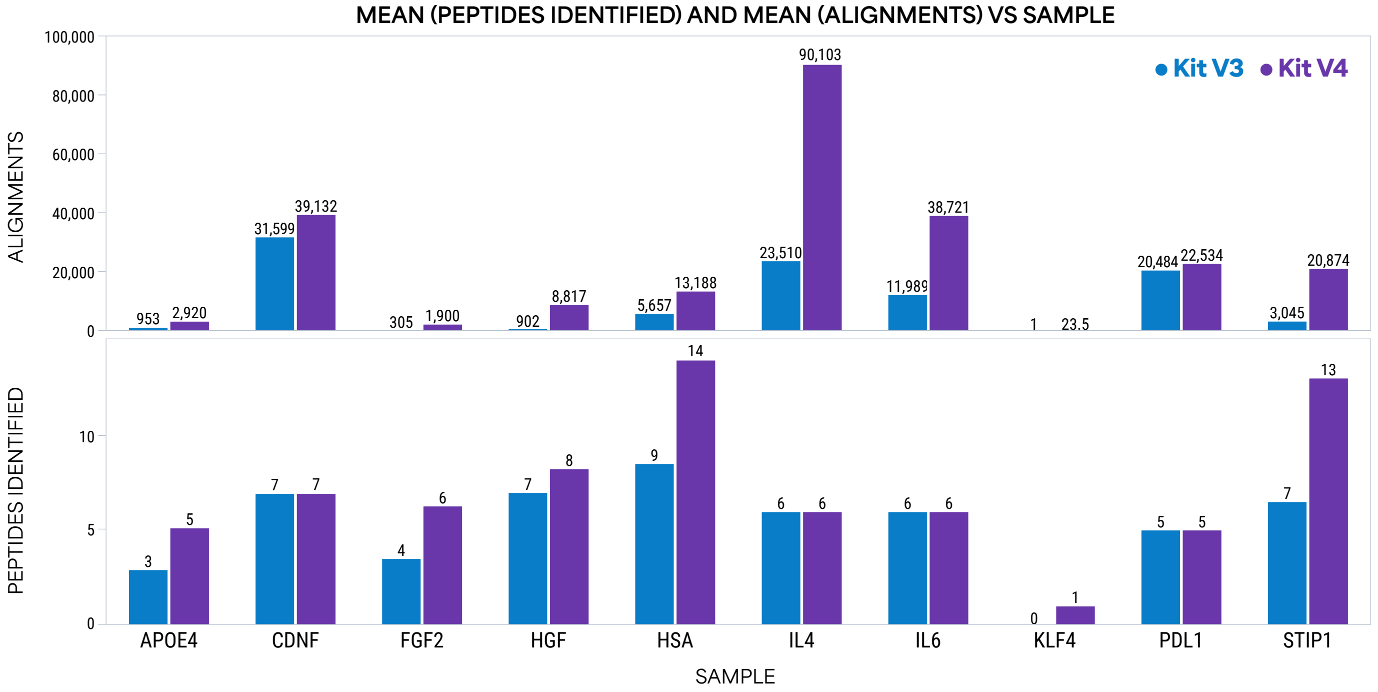
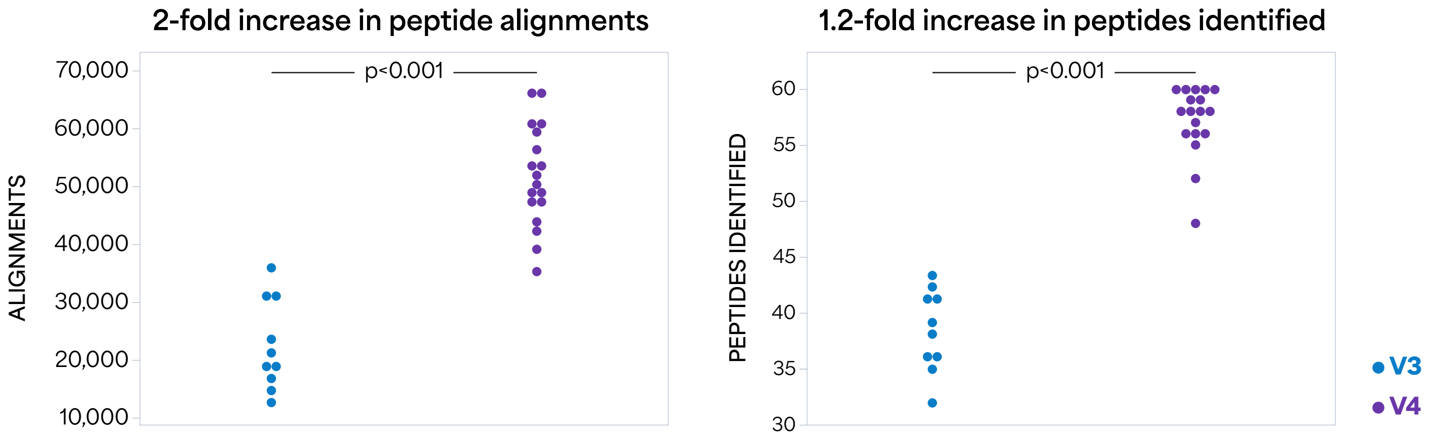

MEAN (PEPTIDES IDENTIFIED) AND MEAN (ALIGNMENTS) VS SAMPLE

Figure 2. Sequencing ten recombinant proteins, from 15 to 66 kDa, demonstrates that the V4 kit produces more peptides and alignments than the V3 kit, with several peptides detected only by V4. V3 sequencing data was analyzed with analysis software v2.15, and V4 was analyzed with software v2.18, optimized for recognizers provided in Sequencing Kit V4.

Protein Mixture Precision

Upgrades to the analysis software boost confidence in accurate protein identification in mixtures using V4. An equimolar mixture of ten proteins sequenced with V4 demonstrated increased peptide alignments, resulting in increased precision in peptides identified compared to V3.


2-fold increase in peptide alignments

1.2-fold increase in peptides identified

Figure 3. Upgrades to the analysis software boost confidence in accurate protein identification in mixtures using V4 when comparing an equimolar mix of ten proteins of different sizes. V3 sequencing analyzed with software v2.15, and V4 with software v2.18.

Sequence Antibodies

Prolines play a crucial role in antibody structure and function, shaping complementarity-determining regions (CDRs) and immunoglobulin loops. We sequenced trastuzumab, an anti-HER2 monoclonal antibody used in cancer treatment, on the Platinum Pro, to demonstrate the improved coverage obtainable with V4, exhibiting:

- **2.5-fold more peptide coverage** in heavy and light chains compared to V3
- **Significant increase in amino acid calls** after proline with V4 chemistry

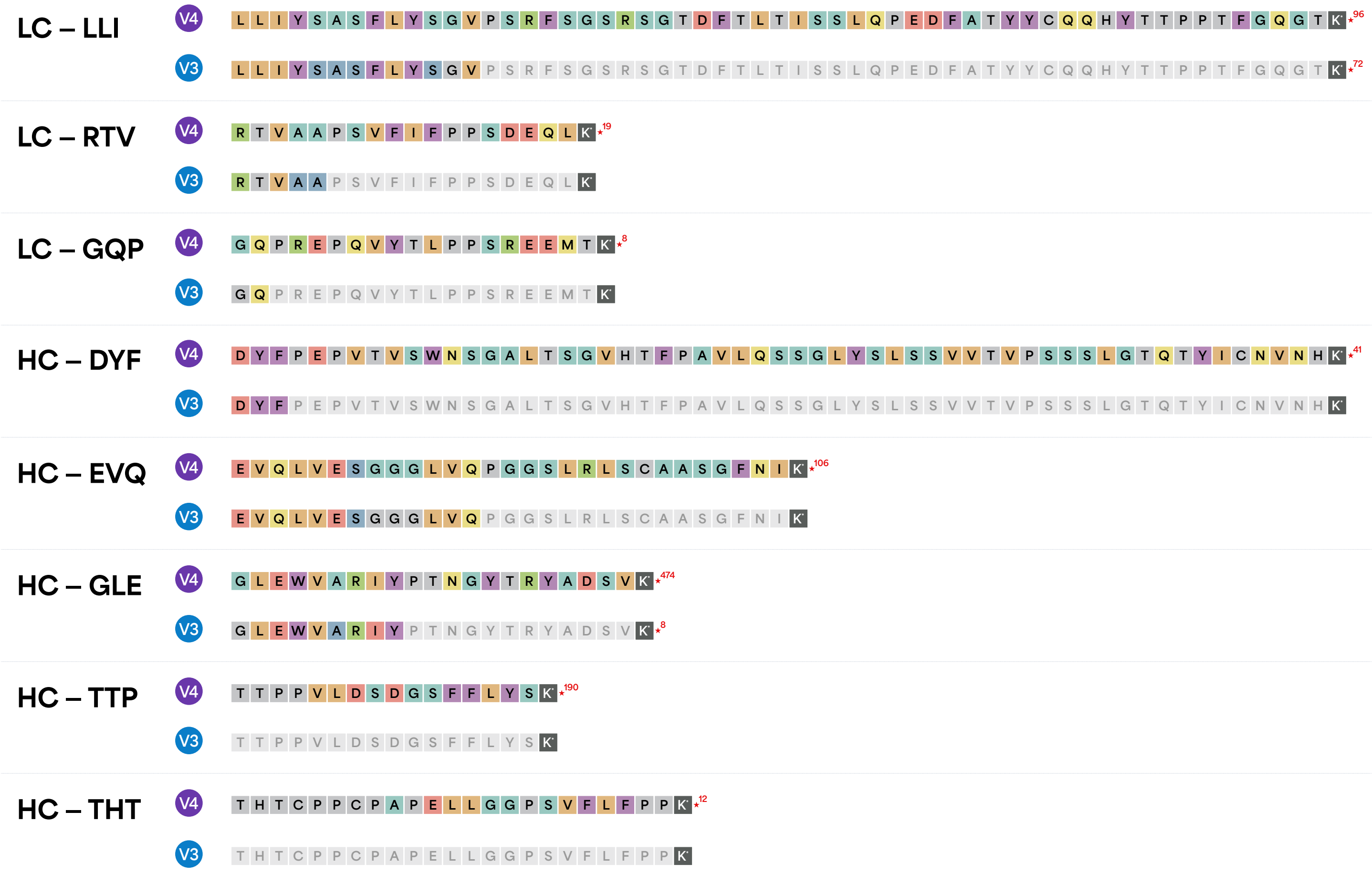
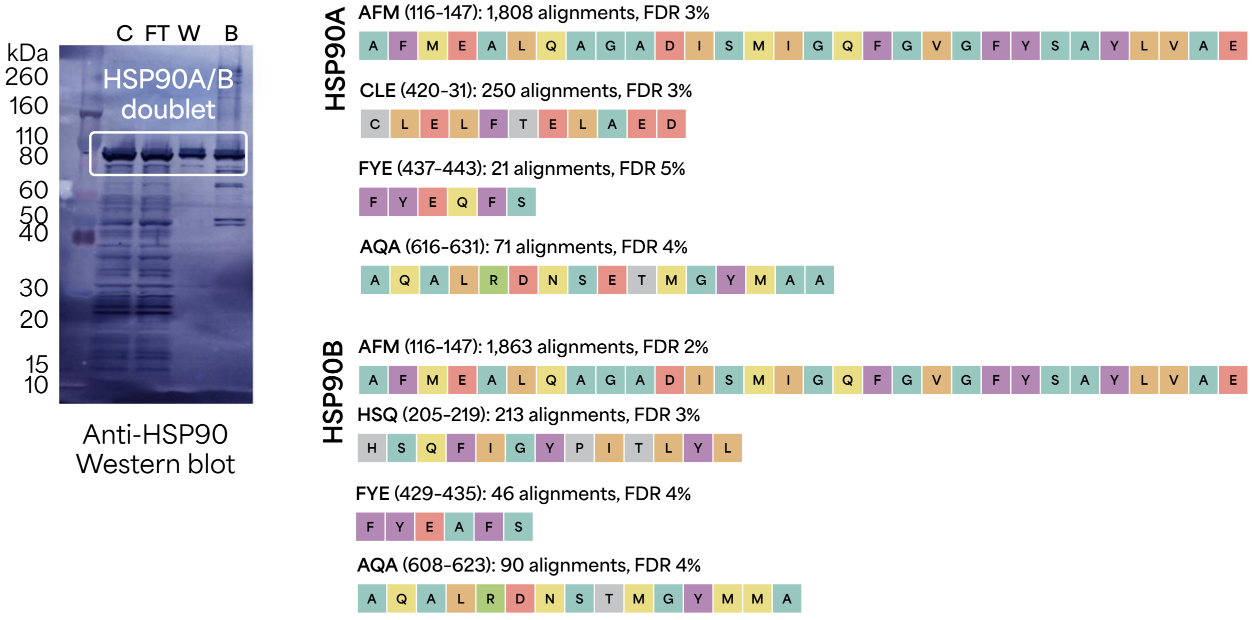


Figure 4. Sequencing of light and heavy chains of trastuzumab with V4 and *Binder Order* sequencing workflow v2.18 demonstrates a significant increase in amino acid coverage obtained through glycine (G) and methionine (M) recognition, enhanced alanine (A) and serine (S) recognition, and identification of amino acids beyond proline (P) compared to Sequencing Kit V3 analyzed with software v2.15. LC: light chain; HC: heavy chain.

Characterize Proteins by Immunoprecipitation

Traditional methods for identification of proteins enriched by immunoprecipitation (IP) vary in their resolution, where Western blot offers qualitative to semi-quantitative measurements that are antibody-dependent, and mass spectrometry can produce exhaustive data inclusive of proteins that may be nonessential to a study. Platinum Pro with the V4 kit is an accessible non-antibody-dependent, higher-resolution option optimized to identify target proteins with concise data readouts.

- **Sequence IP products** on Platinum Pro for clear identification of protein interactors
- **Explore signaling pathways**, antibody binding partners, and other protein interactions



Protein	Inference Rank	Probability
HSP90A	1	99.99%
HSP90B	2	99.96%

Figure 5. Heat shock protein (HSP90) enriched from MCF-7 cells, sequenced with V4, and analyzed with *Binder Order* sequencing workflow v2.18 correctly identified HPS90 A and B as top-ranked inference with an FDR <10% using the whole human proteome. C: crude; FT: flow-through; W: wash; B: beads.

Conclusions

The Platinum Pro instrument and Sequencing Kit V4 deliver real-time single-molecule sequencing data with greater confidence, enabling analysis of proline-rich regions that were previously inaccessible to mass spec. The upgraded analysis software enhances peptide identification accuracy, especially for proline-containing peptides, broadening amino acid recognition. Compared to V3, V4 offers more complete peptide coverage and better alignment. Sequencing Kit V4 allows for a more comprehensive view of the proteome, making high-confidence protein sequencing achievable for both targeted and mixed samples.

Ordering Information

Product	Catalog number
Platinum Pro instrument	910-10904-00
Sequencing Kit V4	910-00038-04
Library Preparation Kit, V2, Lys-C	910-00012-02
Barcoding Kit	910-00047-00

www.quantum-si.com/seq-kit