# Quantitative and precise plasma proteome analysis with enhanced coverage

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## INTRODUCTION

Plasma is a valuable source for the discovery of biomarkers, as it contains proteins such as cytokines and tissue leakage markers that reflect the health status of patients. However, mass spectrometry (MS)-based plasma proteomics is challenged by the vast dynamic range of protein concentrations and signal suppression from high-abundance proteins, which can obscure low-abundance analytes. Enrichment strategies improve coverage but often distort the original protein concentrations, making it difficult to interpret the quantification. The new combined P2 paramagnetic nano-particle and iST technology further extends proteome coverage while preserving quantitative accuracy. In this study, we evaluated its performance using spike-in experiments with different plasma sources.

## MATERIALS & METHODS

**Samples:** A species dilution experiment was performed by mixing K2EDTA bovine plasma (Neo Biotech) with pooled K2EDTA human plasma (Proteogenex) at seven defined volume ratios (1:0 to 0:1), each in triplicate.
For protein-specific spike-in experiments, purified IL-6 (Absea, cat. PP-496), IL-1β (Absea, cat. PC-490), and MAPT (Absea, cat. PC-006) were added to 100 µL K2EDTA human plasma to generate a five-fold serial dilution series from 1250 ng to 0.4 ng, each in triplicate.
**Sample preparation:** Neat plasma (2 µL) was processed using the iST-BCT protocol. P2-iST Plasma samples were prepared from 100 µL plasma on a KingFisher™ Flex, including enrichment, lysis, digestion, and peptide cleanup.
**LC–MS analysis:** 800 ng peptides were analyzed using the Evosep™ One system with Evotip Pure™ and an Aurora Elite CSI 75 µm × 150 mm C18 column, coupled to a timsTOF HT mass spectrometer operating in diaPASEF® mode (Whisper™ Zoom 40 SPD).
**Data processing:** Raw files were analyzed in Spectronaut® 20 using Pulsar-generated spectral libraries; fold changes were derived across the dilution series. Specific normalization and noise filtration strategies were applied.

## KEY TAKEAWAYS

**High precision at greater depth**
P2-iST Plasma markedly expands proteome coverage while maintaining low variability, enabling precise quantification for many more proteins than neat plasma.

**Accurate relative quantification**
Fold-change accuracy is preserved across multispecies dilutions and spike-ins, with quantitative performance comparable to neat plasma at the proteome level.

**Linear signal–concentration relationship**
P2-iST Plasma provides robust linearity and improved LOD/LOQ across a broad concentration range, supporting both discovery and targeted proteomics.

## CONTACT & MORE

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**Conflict of Interest Disclosure**
Grey, M. is employed by PreOmics Inc.
Hu, Z., Limm, K. are employed by PreOmics GmbH.
Schär, S., Arthur, V., Bruderer, R. are employed by Biognosys AG.

**References:**
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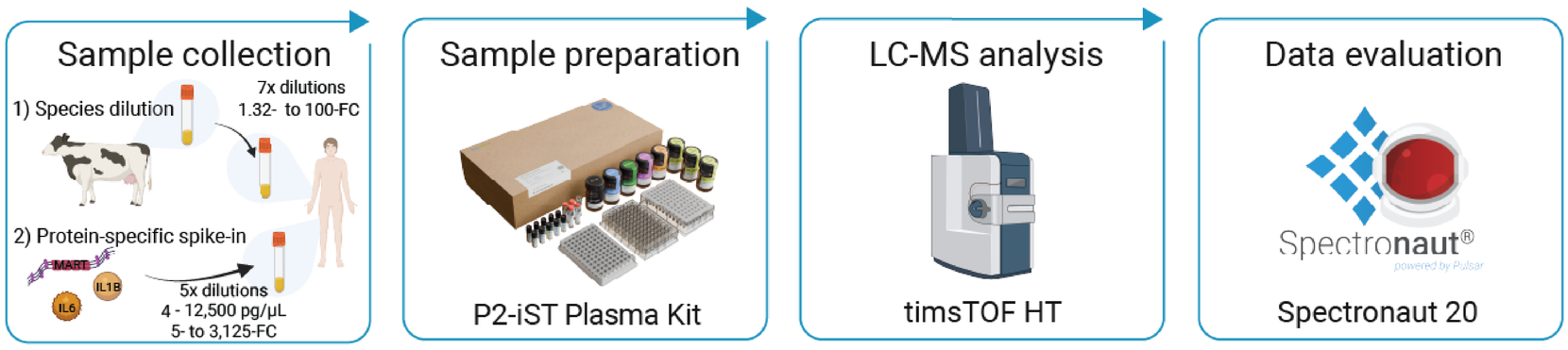
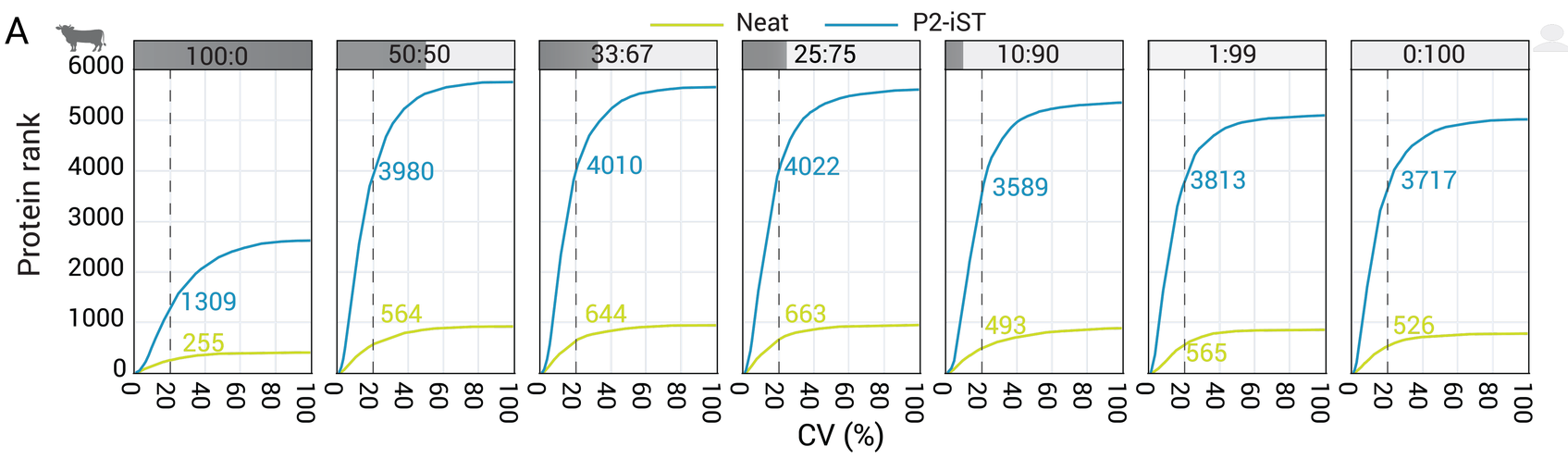
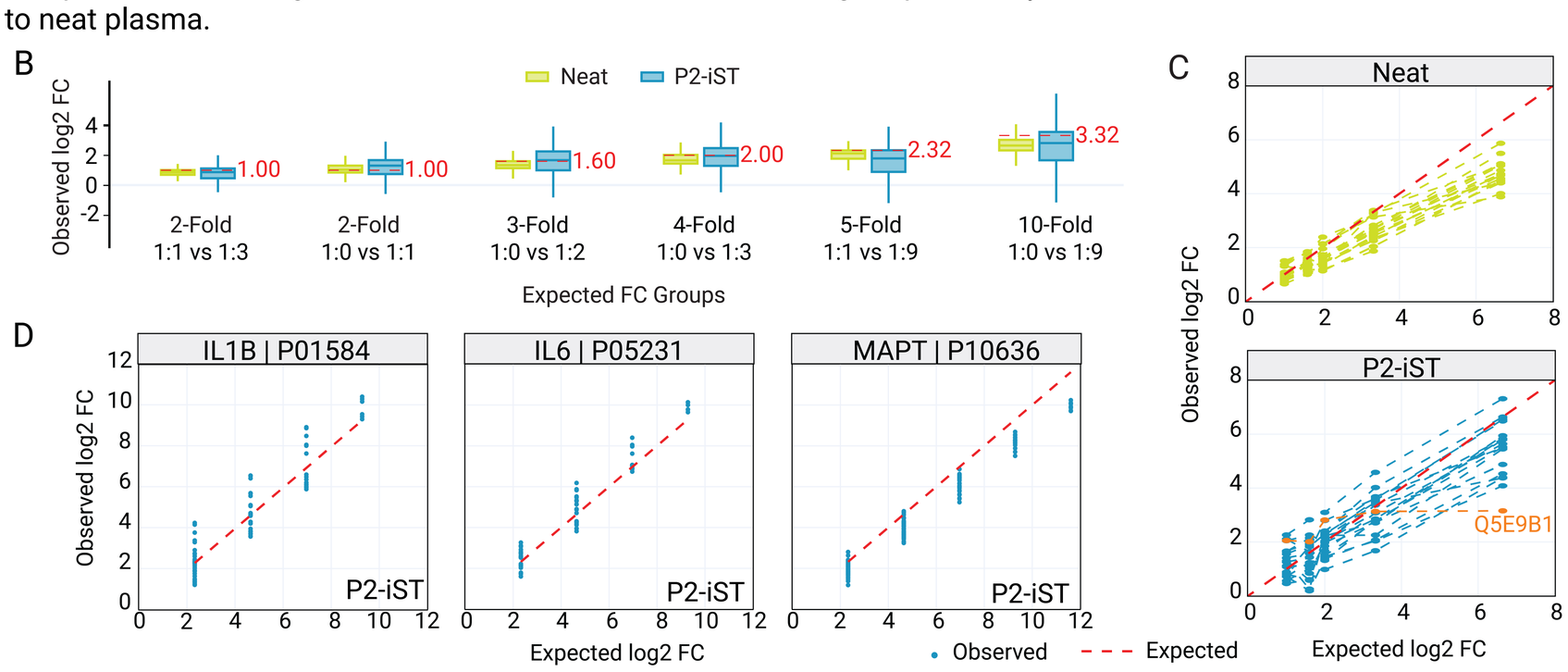
## RESULTS



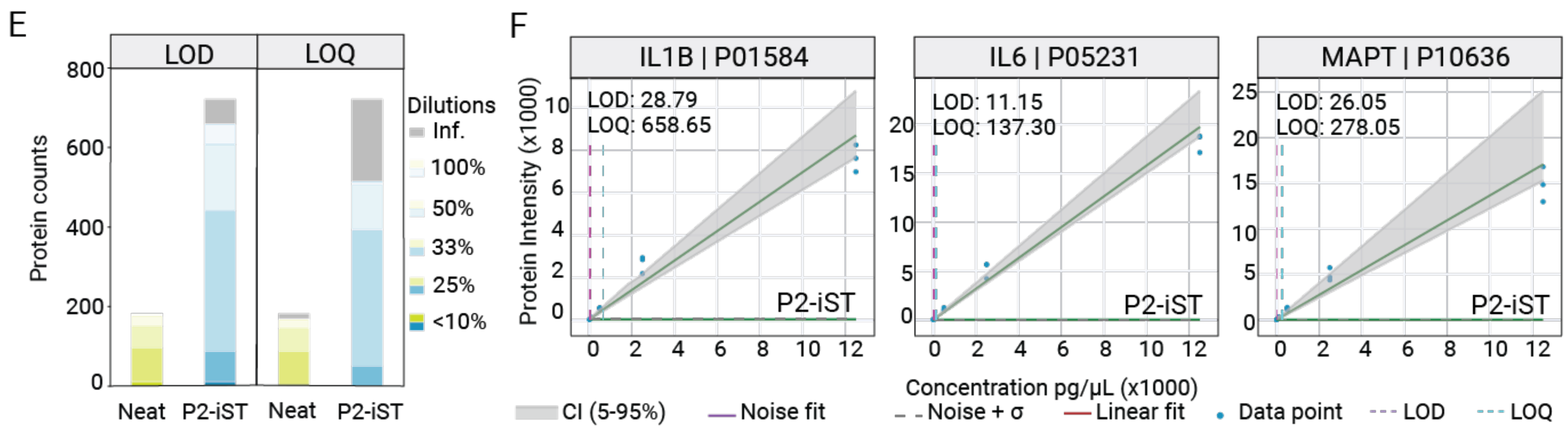

**Experimental design of (1) species dilution and (2) protein-specific spike-ins for benchmarking the quantitative performance of the P2-iST Plasma workflow.**




**Enhanced proteome coverage and precision using P2-iST Plasma in a species dilution study.** (A) P2-iST Plasma enables exceptional plasma dynamic range (7–8 orders of magnitude) by enriching low-abundance proteins, increasing the number of precisely quantified proteins (CV <20%) by 5–7-fold compared with neat plasma, depending on species and mixing ratio. These results demonstrate the high repeatability of the P2-iST Plasma workflow relative to neat plasma.




**High relative quantitation accuracy demonstrated by P2-iST Plasma.** (B) Both P2-iST Plasma and neat plasma samples show high fold-change (FC) accuracy and strong quantitative concordance at the full-proteome level. (C) Fifteen commonly detected FDA-approved biomarker bovine orthologues exhibit accurate FC trends in both workflows, with a single outlier (LDHB, Q5E9B1) in the P2-iST Plasma dataset, potentially attributable to carryover from randomized acquisition order or high sequence conservation between bovine and human proteins. (D) Protein-specific spike-in responses for IL1B, IL6, and MAPT show consistent and accurate FCs derived from pairwise dilutions, confirming that P2-iST Plasma supports robust quantitative measurement of cytokines and key biomarkers relevant to neurodegenerative diseases.




**High linearity delivered by P2-iST Plasma.** (E) Matrix-matched, calibration-based LOD/LOQ analysis demonstrates that P2-iST Plasma achieves higher sensitivity and improved linearity compared with neat plasma, enabling detection and quantification of ~3.5-fold more proteins, particularly at intermediate dilution levels. (F) Spiked-in cytokines IL1B and IL6, as well as the biomarker MAPT, are detectable at tens of pg/µL due to low background noise and are quantitatively measured from hundreds of pg/µL up to at least 12.5 ng/µL with a highly linear response, highlighting the suitability of P2-iST for targeted proteomics applications.