

The iST method provides significant time savings as complex workflows previously requiring many different reagents taking 48 hours are replaced by a single kit containing all standardized reagents. The iST kit allows sample preparation for mass spectrometry-based protein analysis, whether manually, semi or fully automated, in a 2 hour time window. The iST kit is compatible with a wide range of input materials, such as cells, biological fluids, intact model organisms, mammalian and plant tissues or protein-based samples like immunoprecipitations or precipitated proteins.

We demonstrate excellent reproducibility and high protein identifications over a wide protein input range of 1-100 μg and excellent quantitative reproducibility when processing undepleted serum (Figure 1).

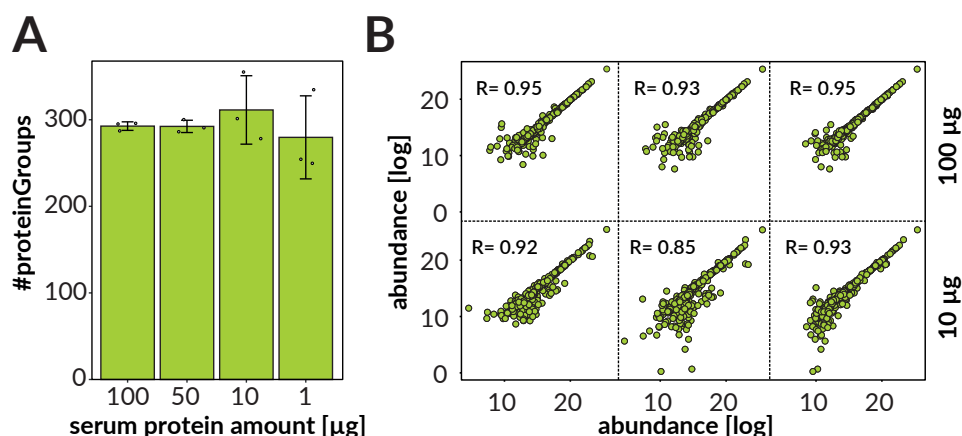


Figure 1 | iST for serum

(A) Dilution series of human serum, proteinGroup identifications when processing either 100, 50, 10 or 1 μg of protein starting material. (B) Quantitative reproducibility of triplicates from (A) for 100 or 10 μg serum input. Triplicate analysis, data acquisition on QExactive Plus.

In combination with mechanical force disruption methods such as bead milling, liquid nitrogen grinding or ultrasonication, the iST kit is perfectly suited to process more complex sample matrices as mammalian and plant tissues too. We show processing of three different mammalian tissue types (brain, liver and heart) using ultrasonication and downstream peptide fractionation by high pH (Figure 2A). Finally, we demonstrate high protein identifications combined with liquid nitrogen grinding when processing plant tissues grown under environmental stress conditions (Figure 2B).

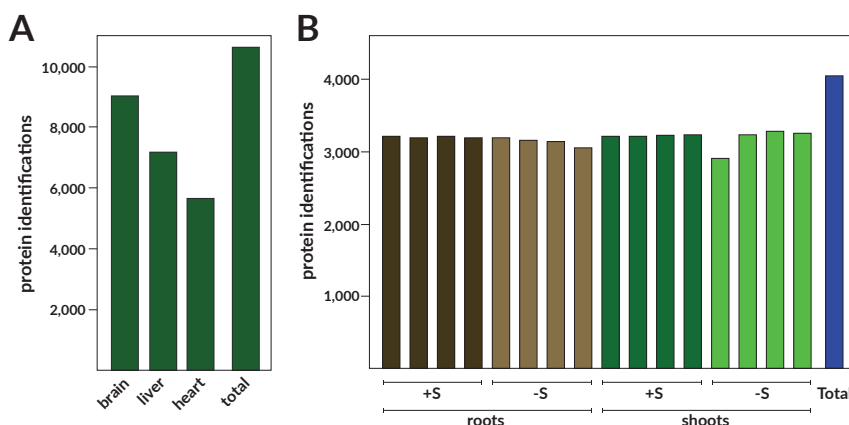


Figure 2 | iST for mammalian and plant tissues

(A) Protein identifications of three different tissue samples fractionated using high pH (8 fractions). Data acquisition on QExactive HF-X. (B) Protein identifications from *A.thaliana* root and shoot tissue in presence or absence of sulfur during plant cultivation. Data acquisition on LTQ-Orbitrap XL.