



Variant-specific Quantification of Therapeutic Targets

Raffaella Berger¹, Anika Kötemann¹, Serhat Beyaz¹, Sandra Poser¹, Viktoria Fischer¹, Theresa Keil¹, Christin Zasada¹, Hannes Hahne¹ ¹Momentum Biotechnologies, Freising, Germany

Background: Efficacy, durability, and safety of next-generation therapies such as RNA-, cell- and gene-based therapeutics, ultimately depend on (often subtle) changes in protein expression and isoform- or mutation-specific selectivity. Consequently, their successful development requires highly precise quantification of proteins alongside their individual variants, many of which remain inaccessible to conventional antibody-based detection methods. QuantScout™ is a targeted, PRM-based mass spectrometry platform that enables highly specific, sensitive and robust quantification of multiple protein targets across diverse biological matrices. **Method:** For the evaluation of therapeutics aimed at modulating erythroid differentiation or globin expression, the precise measurement of fetal vs. adult globin chains, total hemoglobin abundance and subtle shifts in globin composition is crucial. To address this need, we leveraged the minor sequence differences between α -, β -, and γ -globin subunits to identify subunit-specific peptides and developed a quantitative QuantScout™ assay for their simultaneous measurement. The use of heavy isotope-labelled reference peptides further enabled absolute quantification of protein abundance and globin subunit ratios. **Results:** The established assay enabled sensitive detection and precise discrimination between fetal and adult hemoglobin, measuring γ -globin (HBG - fetal hemoglobin), β -globin (HBB - adult hemoglobin) and α -globin (HBA - total hemoglobin reference). Comparing the abundance of γ and β globin subunits in human blood demonstrated (expectedly) low expression levels of fetal hemoglobin in adult samples. The high-confidence, reproducible quantification of hemoglobin subunits allowed for the detection of subtle abundance differences for the direct assessment of disease states and therapeutic effects, even in the low abundance range. **Conclusion:** QuantScout™ provides a highly sensitive, quantitative and scalable targeted proteomics solution for therapeutic development. The ability to precisely quantify disease relevant proteins and highly homologous isoforms in complex biological settings makes it also particularly well suited for next-generation RNA-, cell- and gene-therapy applications.