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Targeted MS quantification assays for signal transduction protein pathways

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Protein quantification expressing their differential expression is underpinning the efforts to identify potential biomarkers. The fact, that there could be hundreds of proteins in a signaling pathway or forming a particular protein class of interest (such as kinases), puts strains on development of targeted assays using immunoassays. The utilization of SRM assays on proteotypic peptides used as 'surrogates' for proteins of interest provides an equally effective approach for protein quantitation.

The selection of a suitable proteotypic peptide is, however, far from straightforward. So far, i*n silico* predictions remain inferior to the empirical peptide strategy. In addition to the obvious concerns regarding adequate selectivity and sensitivity, issues arise with respect to 'real protein' characteristics, such as modifications, folding, digestion efficiency. Using a recombinant protein of interest would resolve most of the above mentioned issues.

The Thermo Scientific Pierce Human *In Vitro* Protein Expression System (IVT) is a method for expressing proteins from DNA or mRNA templates in a cell-free solution containing essential components of the cellular translational machinery. Extracts of an immortalized human cell line provide the ribosomes, initiation and elongation factors, tRNAs and other basic components required for protein synthesis. When supplemented with proprietary accessory proteins, ATP, and an energy regenerating system, such an IVT can produce proteins for use in the mass spectrometry assays in two and a half hours. Human proteins expressed using Heavy IVT system show higher than 90% heavy isotope incorporation. Moreover, proteins are produced appropriately folded and modified, as tested for expressed phosphorylated or glycosylated proteins.

In vitro protein expression system combined with SRM represents a scalable strategy for targeted peptide selection. It will be discussed in more detail for a subset of transcription factors representing various signal transduction pathways.