

Sección temática: PROTEÓMICA

Título: Selected Reaction Monitoring (SRM) frente a Western Blot.

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We propose the SRM technology as a complementary method to the Western Blot for the detection and quantification of proteins in a sample.

The technique Western Blot has its own limitations: i) only a protein-of-choice is detected, ignoring any non-relevant proteins, ii) the sensitivity of the technique depends on the specificity of the antibody and iii) Western Blot is expensive and time-consuming.

The advantages of SRM with respect Western Blot are remarkable: i) you can detect up to hundreds of different proteins in a sample, ii) SRM is more sensitive, because just 50 copies of the target protein per cell are enough for the detection and iii) once it has been made an investment in the necessary machinery to develop this technique, the detection of proteins in a sample turns into a cheaper, faster, more specific and full-quantitative procedure, without the need of using antibodies.

First of all, SRM requires the identification of little peptides, obtained by tryptic digestion, whose sequence must be unique for a single protein or isoform. There is software for that aim. Then, it's necessary to create isotope-labeled peptides of that identified for acting as internal standards. That sample is introduced in a triple quadrupole mass spectrometer: it passes through a first quadrupole, which functions as a filter, where the fragments are selected, previously ionized, attending to the mass/charge (m/z) relation that correspond to that unique fragments of the protein of interest. In this first selection may be other peptides from other proteins, with the same m/z but with different sequence. To select those that are exclusive from the target protein, the fragments are moved to a second quadrupole, where they are fragmented again with a physical method, and so new smaller fragments are generated. All the new fragments are conducted to the third quadrupole, where just those which come from the protein of interest are selected, attending at their m/z again. The target peptide concentration is determined by measuring the observed signal response for the target peptide relative to that of the isotopic-labeled peptide, the concentration of which is calculated from a pre-determined calibration-response curve. Calibration curves have to be generated for each target peptide in the sample.

Because SRM technology is increasing its use, there have been developed databases where the scientific community upload information about protocols and standards for each protein with the aim to facilitate the work to other researchers.