Teaming Seppro® and iTRAQ® for Biomarker Discovery

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In proteomics, protein depletion technologies, such as Seppro from Sigma-Aldrich®, have proven useful in increasing sample dynamic range via removal of high-abundance proteins that are not necessarily of immediate biomedical or clinical relevance. This is of particular importance in the context of biomarker discovery, where putative protein biomarkers are often present at low levels and thus are easily masked by highly abundant proteins.[1] After depletion of high-abundance proteins, tagging chemistries like iTRAQ, mTRAQ® and ICAT® from AB Sciex are available to label, isolate, and analyze the remaining protein pool to capture potentially useful biomarkers,[2] which can undergo further investigation with multiple reaction monitoring (MRM) studies.[3] In their systematic study of plasma sample analysis using iTRAQ, Ohlund et al. discussed the use of various protein depletion technologies, including Seppro, in conjunction with iTRAQ.[4]

Several very recent publications have looked at the tandem use of Seppro and iTRAQ in biomedical work. Müller et al. have studied the human skin suction blister fluid (SBF) proteome as a possible biomarker source for skin-related disorders. The authors tested two immunodepletion technologies, including Seppro IgY14, and combined immunodepletion with downstream iTRAQ treatment. This proof-of-principle investigation was the first publication on a gel-free, quantitative MS study of the SBF proteome.[5] Rehman, Evans and colleagues looked at human serum prostate cancer samples to probe for potential novel biomarkers, also with Seppro IgY14 and iTRAQ used in tandem. Their study revealed several potential candidate proteins, such as eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) and afamin.[6] Zhang et al. have investigated plasma samples from colorectal cancer (CRC) patients, using a workflow where they teamed Seppro technology with iTRAQ. They identified 13 potential CRC biomarkers, such as ORM2 and serpin peptidase inhibitor, clade D.[7]

Sigma-Aldrich R&D has recently collaborated with researchers at Fox Chase Cancer Center, Temple University, and National Jewish Medical & Research Center in a more novel combination of the Seppro and iTRAQ technologies in a single workflow. This newly published study extends the range of the Seppro-iTRAQ coupling by using both the IgY14 and the SuperMix Seppro technologies together, in order to deplete both the 14 most abundant proteins and moderately abundant proteins from plasma.[8] In principle, this additional level of protein depletion can enhance further potential detection of the lowest abundance proteins from plasma, although concerns do exist about ancillary depletion of proteins that can be potential biomarkers.[4] The publication, by Patel et al., looked at such concerns and noted more quantitatively the need to exercise caution in designing immunodepletion experiments, to factor in the possible association of low-abundance, potential biomarker proteins with immunodepleted, higher abundance proteins. This is consistent with earlier studies by Qian et al. on comparable Seppro tandem systems of IgY12 or IgY14 plus SuperMix (without subsequent iTRAQ treatment), where the authors advised that both the flow-through and bound protein fractions in immunodepletion should be evaluated in quantitative proteomics research.[9,10]

Sigma-Aldrich has partnered with AB Sciex to offer the iTRAQ product line as part of the Sciex iChemistry portfolio of reagents for quantitative proteomics. The iChemistry portfolio also includes the ICAT and mTRAQ tagging reagents. With product lines such as Seppro and iChemistry now available, Sigma-Aldrich provides you the necessary tools for the complete proteomics workflow.

References

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