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HIGH THROUGHPUT PROTEOMIC ANALYSIS USING DIFFERENT OFFGEL FRACTIONATION PANELS

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OFFGEL fractionation of mouse kidney protein lysate and its tryptic peptide digest has been examined in this study for better understanding the differences between protein and peptide fractionation methods and attaining maximum recruitment of this modern methodology for in-depth proteomic analysis. With the same initial protein/peptide load for both fractionation methods, protein OFFGEL fractionation showed a preponderance in terms of protein identification, fractionation efficiency, and focusing resolution, while peptide OFFGEL was better in recovery, number of peptide matches, and protein coverage. This result suggests that the protein fractionation method is more suitable for shotgun analysis while peptide fractionation suits well quantitative peptide analysis [isobaric tags for relative and absolute quantitation (iTRAQ) or tandem mass tags (TMT)]. Taken together, utilization of the advantages of both fractionation approaches could be attained by coupling both methods to be applied on complex biological tissue. A typical result is shown in this article by identification of 8262 confident proteins of whole mouse kidney under stringent condition. We therefore consider OFFGEL fractionation as an effective and efficient addition to both label-free and quantitative label proteomics workflow.

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