

Phosphopeptide enrichment – methods, difficulties, biological use

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Phosphorylation is one of the most common post-translational modifications of proteins and has important biochemical roles. It takes part in the fast on/off switch of enzymes and plays a role in the regulation of synaptic signal transmission.

In contrast to the unphosphorylated peptides, due to the large variety and low concentration of phosphopeptides, their qualitative determination and quantification is a difficult analytical task. For this reason, additional phosphopeptide enrichments are required for their quantification.

In addition to shotgun proteomic experiments, sample preparation for phosphorylation analysis must be started with a C18 SPE sample cleanup of the digested samples. This is inevitable for the elimination of impurities and metal ions from the sample. Therefore, first we performed a detailed examination for the loading capacity of C18 SPE columns.

Using a set of model proteins and a phosphopeptide standard mix, we determined the maximum enrichment efficiency for TiO₂ and Fe-IMAC SPE columns. During the optimization of experimental conditions it was found that the use of more acidic loading and washing puffer solutions was more advantageous.

We applied the optimized TiO₂ enrichment protocol to study the effects of sleep deprivation on the mouse synaptosome. Four sample groups were analyzed modelling sleep deprivation and recovery sleep. The results show extensive changes pertaining the trafficking of synaptic vesicles and synaptic metabolism.