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Protocol status: Working
We use this protocol and it's working

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Proteomics :On-bead/in-solution digestion and phosphopeptide enrichment

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ABSTRACT

Proteomics :On-bead/in-solution digestion and phosphopeptide enrichment

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1 Protein digestion of GST samples was performed following published protocols

CITATION

Soucek S, Zeng Y, Bellur DL, Bergkessel M, Morris KJ, Deng Q, Duong D, Seyfried NT, Guthrie C, Staley JP, Fasken MB, Corbett AH (2016). The Evolutionarily-conserved Polyadenosine RNA Binding Protein, Nab2, Cooperates with Splicing Machinery to Regulate the Fate of pre-mRNA..

LINK

https://doi.org/10.1128/MCB.00402-16

2 Digestion buffer (50 mM NH_4HCO3) was added to the sample, followed by treatment with 1 mM dithiothreitol (DTT, Thermo Scientific Cat#R0861) at room temperature for 30 min. Subsequently, 5 mM iodoacetamide (IAA, Sigma Cat#I1149) was added to the mixture and incubated at room temperature for 30 min in the dark.

3 Proteins were digested with 2 µg of lysyl endopeptidase (Wako, Cat# 125-05061) at room temperature (overnight).

4 Further digestion was performed with 2 µg trypsin (Promega, Cat#VA9000) at room temperature (overnight). In contrast, for phosphopeptide enrichment, proteins were digested overnight at room temperature with 1 µg of Asp-N (Promega, Cat# VA1160) for samples in solution or 5 µg of Asp-N for the samples on-beads.

5 The clean peptides were concentrated using a high-select Fe-NTA phosphopeptide enrichment kit (Thermo Scientific Cat#A32992) All resulting peptides were desalted and dried using an HLB column (Waters, Cat#186002034) and vacuum.