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Optimization of expression, purification and HRMS characterization of recombinant N-protein fragment from SARS-CoV-2

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Nucleocapsid (N) protein is the most abundant SARS-CoV-2 virus derived protein and strong immunogen which can be used as a component of the immunological tests for the diagnosis of SARS-CoV-2 infection. Recombinant fragment of N-protein (58–419 aa) was expressed in *E. coli* in a soluble form using developed optimized protocol of expression (16-18h, 37 °C, 0.4 mM IPTG). After lysis of cells, N-protein from soluble fraction of lysate was purified using optimized protocol for purification by immobilized metal affinity chromatography on Ni-Sepharose in two repeated steps under different elution conditions. Obtained fraction of N-protein after the second chromatography was desalted and concentrated using phosphate buffer solution and ultrafiltration. The purity of isolated N-protein was deterrmined by SDS PAGE, while high resolution mass spectrometry was used for its characterization. Isolated N-protein was over 90% purity and identified as the most intense and abundant protein fragment, with PEAKS PTM score of 508 and sequence coverage of over 70%, including 173 unique peptides.

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