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Proceedings

Editor: Ivan Spasojević

Technical support: Dragana Robajac

Cover design: Zoran Beloševac

Publisher: Faculty of Chemistry, Serbian Biochemical Society

Printed by: Colorgrafx, Belgrade

Serbian Biochemical Society

Tenth Conference

with international participation

24.09.2021. Kragujevac, Serbia

“Biochemical Insights into Molecular Mechanisms”

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 determined 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.

Acknowledgements

This research was supported by the Science Fund of the Republic of Serbia, #GRANT No7542203, COVID-19 – CAPSIDO; the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No 451-03- 68/2020-14/200168.

References

1. Djukic T, et al. Expression, purification and immunological characterization of recombinant nucleocapsid protein fragment from SARS-CoV-2. *Virology* 2021;557:15–22.