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SARS-CoV-2 Spike (S) Glycoprotein Expression, Purification and Characterization in Suspension Human Embryonic Kidney Cells 293

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SARS-CoV-2 is a zoonotic virus RNA positive, which became responsible to be the largest sanitary crisis faced by humanity: Coronavirus disease 2019 (COVID-19). Some symptoms include major sneeze conditions, who could evolve to severe acute respiratory syndrome, and in some cases, to death. Techniques for accurate detection of this virus are essential to promote an accurate diagnosis of infected patients. SARS-CoV-2 has several targets with clinical interest; although, the focuses is on Spike (S), a homotrimer glycoprotein, that interacts with angiotensin converting enzyme receptor (ACE2), developing the infection in host cells. Thus, we recognize that the demand for the glycoprotein S is necessary, requiring large amounts with high purity level. The current work has the main objective the transient expression of SARS-CoV-2 S protein into suspension human embryonic kidney cells 293 (HEK293), purification and characterization, to use it as a template for discovering new molecular markers. SARS-CoV-2 S modified protein cDNA was inserted into pαH plasmid, amplified, and purified. For transient recombinant protein expression, 7.5 x 10^7 HEK293 cells (Expi293F™ cells) was seeded in 27 mL Expi293™ culture Medium. The transfection was carried out with a cationic lipid ExpiFectamine™ and 30 μg of plasmid, mixed with 3 mL Opti-Mem® culture medium. Cell culture was maintained for seven days in 125 mL vent cap Erlenmeyer, 32 ºC, 8% CO_2, under 125 rpm orbital shaker rotation. 10 mL aliquots were collected on four- and seven-day post transfection, stored at -80 ºC. Physical-chemical and biological characterizations were determined by SDS-PAGE, ELISA, and Western Blotting. Purification from 40 mL of conditioned medium was carried out in two steps: Strep-Tag affinity column, followed by a size exclusion Superose® 6 (10/300), 5.0 mg of oligomeric recombinant protein with 95% purity was obtained. We believe that this process can be easily adapted to different volumes, being very useful for obtaining, in a short time, enough pure and immunological active SARS-CoV-2 S for further studies and applications, such as, cryogenic electron microscopy, mass spectroscopy, N-glycan structures, antibody production and immunologic assays development.