Electronic Supplementary Information

Electrochemical immunosensor for the detection of staphylococcal enterotoxin B using screen-printed electrodes

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Preparation and affinity purification of SEB antigen

SEB antigen was produced by S. aureus strain ATCC14458 in the biotechnology division of our establishment. Under optimized conditions, the toxin gene was amplified for cloning and expression. To achieve high yield of recombinant SEB, strain of Escherichia coli (E. coli) SG13009 (pREP4) was selected as the host (kanamycin resistant). For affinity purification, the expressed r-SEB was tagged with 6 x histidine as per manufacturer’s instructions (Qiagen, Germany) and cloned in the pQE30UA vector (ampicillin resistant). Recombinant-SEB i.e. (r- SEB) was purified by affinity chromatography method. For this purpose, the cell pellet was resuspended in Ni-NTA buffer (1:10 w/v, pH 8). The recombinant protein was eluted with Ni- NTA buffer (pH 4.5). The purity of the obtained protein was checked by SDS-PAGE method and the yield was calculated by BCA-protein estimation.

Affinity chromatography based IgG purification and BCA-method of protein estimation

Unpurified rabbit anti-SEB polyclonal IgG and mice anti-SEB monoclonal IgG was purified by affinity chromatography based method as per details given by the manufacturer. In this method, unpurified serum sample was first mixed properly with the binding buffer. After that the “Protein A Cartridge” was washed with regeneration buffer. For this purpose, regeneration buffer was passed through the cartridge at the approx flow rate of 1mL/min. Then the cartridge was equilibrated by binding buffer by passing the binding buffer through the cartridge at the same flow rate. Then, we had loaded the sample-binding buffer mixture by passing it to the “Protein A Cartridge” at the approx flow rate of 0.5mL/min. Binding buffer was passed through the cartridge after the sample loading at the flow rate of about 1mL/min. Desalting cartridge was washed with HEPES buffer by passing it through the cartridge at an approximate flow rate of 1mL/min. Then we had attached the one end of the “Protein A Cartridge” to another end of the desalting cartridge. Then, we had eluted the cartridges with elution buffer by passing it through the cartridges at an approximate flow rate of 0.5mL/minute. Elute was contained the purified IgG at physiological pH. After it, we had detached both the cartridges and regenerate them. “Protein A Cartridge” was regenerated by regeneration buffer by passing it through the cartridge. HEPES buffer was passed through the desalting cartridge and was regenerated. These cartridges are ready and can be used for another affinity chromatographic purification.
(a) Shows the standard graph obtained for the estimation of rabbit anti-SEB IgG and (b) shows for estimation of mice anti-SEB IgG using BCA-protein estimation method.

Bicinchoninic acid protein assay kit and spectrophotometric method was utilized for the estimation of purified rabbit anti-SEB polyclonal IgG and mice anti-SEB monoclonal IgG concentration. For this purpose, bicinchoninic acid (BCA) working reagent was prepared by mixed with 50 parts of reagent A (containing bicinchonic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1N NaOH, pH 11 with 1 part of reagent B (containing 4% (w/v) copper(II) sulfate pentahydrate). In the standard procedure, BCA working reagent was taken in 200µL are mixed with 0, 2, 4, 6, 8, 10µL part of a standard protein (BSA) solution of known concentration. 10µL of water without protein was used as blank. Unknown purified IgG sample was assayed with the known concentration of BSA protein standard. After that, the plate was incubated at 37°C for 30min. In the next step, absorbance was recorded at 562nm and the unknown protein concentration was determined by comparison to the standard curve. It was estimated that 2.4mg/mL IgG is present in rabbit-IgG of SEB and 1.0mg/mL IgG was present in mice-IgG of SEB.