APPLICABILITY OF FLUORESCENCE-BASED IMMUNOBIOSENSOR ON HEAT SHOCK PROTEIN DETERMINATION IN GREEN ALGA *CHLORELLA VULGARIS*

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Members of 70 kDa heat shock protein (HSP70) family are highly conserved molecular chaperons and ubiquitously found in almost all living organisms, acting as crucial protectors of cellular integrity under various types of stress by stabilizing proteins and preventing their denaturation or aggregation [1]. HSP70s play a crucial role in protein homeostasis and stress response induced by environmental stressors (i.e. heat, desiccation, changes in salinity, UV radiation, etc.) or pollutants [2]. Algal organisms are usually investigated in ecotoxicity assays as indicator species of the aquatic ecosystem. HSP70s react quickly to these environmental stressors, thus they can be applied as biomarkers in ecotoxicity assays.
Various techniques have been presented for qualification or quantification of HSP70 proteins in different biological matrices. Methods to determine overall HSP70 levels, its intracellular localization, mRNA levels and functional binding include enzyme-linked immunosorbent assays [3–4], flow cytometry [5–6], immunohistochemistry [7–8], western blotting [9–10], quantitative reverse transcription polymerase chain reaction [11–12], fluorescence polarization [13–14] and immunocytochemistry [15].
In this study, the applicability of a double antibody sandwich enzyme-linked fluorescent immunoassay was investigated in HSP70 determination in *Chlorella vulgaris* green alga species. HSP70 calibration curves were obtained in standard assay dilution and in homogenized green alga samples prepared by sample assay diluent. Calibration curves were fitted by the Rodbard equation in the concentration range of 0–71.4 ng/mL. HSP70 standards were determined by both visual and fluorescent signals. Absorbance (optical density) and fluorescence were measured at 450 nm and 593 nm wavelengths (excitation: 535 nm), respectively.

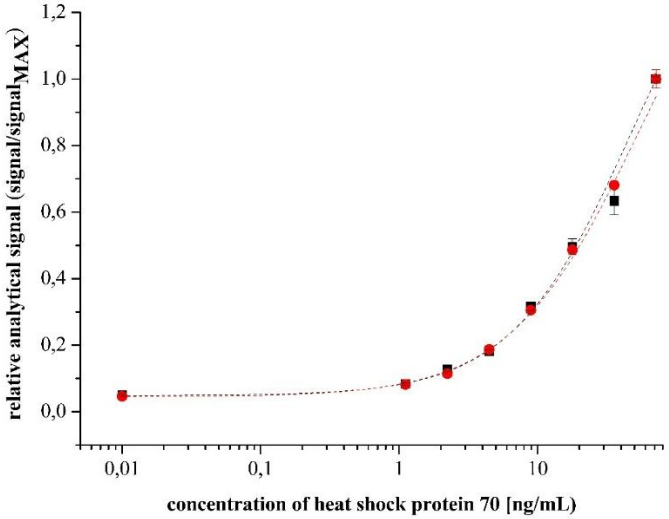

Figure 1. Calibration curves of the double antibody sandwich technique-based enzyme-linked fluorescent immunoassay obtained in assay standard diluent (black) and in homogenized green alga (*Chlorella vulgaris*) sample (red).

Determination by fluorescence provided a wider dynamic range for HSP70 detection. Application of the fluorescent signal provides a 4.5-times lower limit of detection value than that obtained with a visual absorbance signal. Parameters of calibration curves (IC_{50} and slope) did not significantly differed between calibration curves obtained in assay standard diluent and in green alga sample (Figure 1).

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