Analysis of Oxidative Stress Biomarkers Using a Simultaneous Competitive/Non-Competitive Micromosaic Immunoassay

Brian M. Murphy,[a] David S. Dandy,[b] and Charles S. Henry,[a,b]

[a]Department of Chemistry, Colorado State University, Fort Collins, CO 80523

[b]Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO 80523, USA Fax: 970-491-1801; Tel: 970-491-2852;

E-mail cshenry@lamar.colostate.edu

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Abstract

Immunoassays represent a core workhorse methodology for many applications ranging from clinical diagnostics to environmental monitoring. In traditional formats such as the enzyme linked immunosorbent assay (ELISA), analytes are measured singly or in small sets. As more biomarkers are identified for disease states, there is a need to develop methods that can measure multiple markers simultaneously. Immunoaffinity arrays are one such chemistry that can achieve multi-marker screening. Most arrays are performed in either competitive or non-competitive formats, where the former are used predominantly for small molecules and the later for macromolecules. To date, ELISA and immunoaffinity array methods have relied exclusively on one of these formats and not the other. Here an immunoaffinity array method capable of performing simultaneous competitive and non-competitive analysis generated using micromosaic immunoassay techniques is introduced for the analysis of metabolites and proteins. In this report, three markers of oxidative stress were used as a model system. The method described here demonstrates the simultaneous analysis of 3-nitrotyrosine, by indirect competitive immunoassay while the enzymes catalase and superoxide dismutase are analyzed by non-competitive sandwich immunoassay. The method requires less than 1 μL sample and 45 min for completion. Logistic curve fits and LOD statistical analysis of the binding results are presented and show good agreement with published data for these antibody-antigen systems.