Supplementary Material (ESI) for Analyst

SERRS Immunoassay for Quantitative Human CRP Analysis

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Experimental Procedure for Immunoassay and Spectroscopic Analysis

In the assay, 100 μl of a 1:500 dilution of a stock (1mg/ml) solution of biotinylated monoclonal antibody (C2) to human CRP (Biodesign Inc, ME, USA) in 0.1% (w/v) Tween-20 in 0.05 mol/L pH 7.4 tris buffered saline (TTBS) was coated to each well of 96 well Streptavadin coated plates (Thermo Electron Corporation, MA, USA) for 30 min at room temperature (RT) and the plates were then washed for 3x with TTBS. Dilution in TTBS of a purified human CRP (Biodesign) standard gave a range of standards from 100 ng/ml to 0.20 ng/ml. Aliquots (100μl) of the diluted standard, diluted 1:50 in TTBS were added to duplicate wells while 100μl of TTBS was dispensed to duplicate zero control wells. The plates were then incubated for 1hr at RT and washed 3x with TTBS. Thereafter, 100μl of a 1:500 dilution of a stock (1mg/ml) solution of alkaline phosphatase conjugated monoclonal antibody (C6) to human CRP (Biodesign) was added to each well and the plates were incubated for 1hr at RT and then washed 3x with TTBS. A solution of Tris-HCl (0.05M, pH 9.8 containing 0.3mg/ml BCIP and 0.005 M $MgCl_2$) was prepared and 100μl added to each well. After allowing to proceed for 30min at RT, the reaction was stopped by adding 50 μl of 1M H_3PO_4 solution to each well. Then, 100 μl of the reaction solution in each well was transferred to another 96 well plate and 100 μl of gold colloid added before SERRS analysis was carried out. Gold colloid was synthesised by citrate reduction of $HAuCl_4$, affording particles of approximately 13 nm in size. The SERRS spectrum for the solution in each well was acquired using a Leica DM/LM microscope equipped with an Olympus 20x/0.4 long working distance objective to collect 180 º backscattered light from a standard microtiter plate. The spectrometer system was a Renishaw Ramascope system 2000 with a Renishaw HeNe laser as the excitation source (λ_{ex} = 632.8 nm). Power output was measured to be approximately 6 mW at the sample. Dielectric edge filters were used to reject Rayleigh scattered light.