

Picomolar Level Biomarker Detection in Complex Media Using an Indirect Sandwich Assay

Introduction

There is considerable demand for high-sensitivity immunoassays to detect picomolar level analytes in complex biological samples. The ability to detect analytes in a matrix such as plasma over a broad concentration range would lend itself to the detection of many clinically relevant analytes.

Natriuretic peptides play a variety of important physiological roles and also serve as very valuable biomarkers of cardiac function and disease. proBNP is produced in the muscle cells of the left ventricle of the heart. It breaks down into two fragments, B-type natriuretic peptide BNP and the N-terminus, NT-proBNP. BNP is the biologically active form which causes bodily responses, whereas NT-proBNP is a biologically inactive form which can be measured due to its relative stability over time. Physiologically relevant concentrations are usually in the picomolar range.

This study will demonstrate a quantitative approach to picomolar detection of NT-proBNP in plasma. This assay design has general applicability for other analytes at picomolar concentrations.

Materials

Instrument: dotLab™ System¹

Sensor: Streptavidin dotLab Sensor¹

Assay Components:

- Biotinylated mouse monoclonal anti-NT-proBNP (Bt-Ms)
- Recombinant human NT-proBNP (rhNT-proBNP) [obtained from a collaborator]
- Affinity-purified goat polyclonal serum directed against NT-proBNP (Gt-anti-NT)
- Control Plasma: plasma was devoid of NT-proBNP [obtained from a collaborator]
- Enhancement Antibody: Donkey anti-goat conjugated with HRP (Dk-HRP) [Jackson Immuno-Research Laboratory, Cat No. 705-035-14]

- Enhancement: 3,3',5,5' Tetramethylbenzidine (TMB), 1-component TMB membrane peroxidase substrate [Kirkegaard & Perry Laboratories, Cat No. 50-77-18]

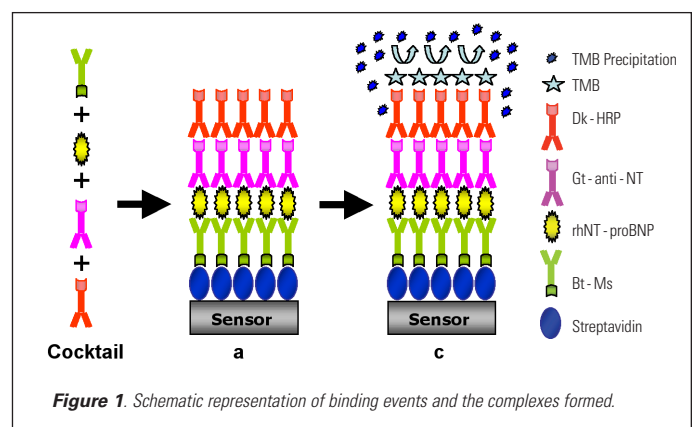
Blocking Agent: Bovine serum albumin (BSA), 10 mg/mL [Axela P/N BM000039], BSA mixed 1:1 with plasma for a final concentration of 5 mg/mL

Buffers:

- Phosphate buffer saline (PBS), 10X [Axela P/N BM000042], filtered and diluted to 1X
- Tween 20, 10% (w/v) [Axela P/N BM000043], diluted to 0.025% in PBS (PBS-T)

Methods

The four-component sandwich ELISA consisted of a cocktail containing all three antibodies and the antigen in a plasma matrix, and was pre-incubated prior to loading. Once loaded onto the sensor, the cocktail was mixed in the channel to maximize binding (see Figure 1). The sensor was then washed with PBS and the TMB precipitating substrate was then allowed to react with the immobilized complexes.



Results and Discussion

Previous optimization experiments had revealed that pre-incubation of the antibodies was necessary to ensure the proper formation of the sandwich (data not shown). A representative real-time binding curve is depicted in Figure 2. The event seen after loading the cocktail reflects the binding of both free and complexed biotinylated monoclonal antibody, Bt-Ms (Figure 2a). During the wash step, the removal of non-specifically bound molecules between the lines of the diffraction grating causes a further signal increase as evidenced in Figure 2b.² TMB precipitating substrate is then allowed to react with the immobilized complexes. The precipitation of TMB increases the diffractive efficiency of the pattern, causing a further increase in signal (Figure 2c). For internal calibration purposes, a ratio was calculated of the signal increase for the TMB precipitation event to that of the cocktail binding event. With this approach, a calibration curve was generated for recombinant NT-proBNP spanning 31.25 - 2500 pg/mL (approximately 4 - 300 pmol/L) in plasma (Figure 3). Note that a ratio of binding rates can also be used, obviating the need to let reactions proceed to equilibrium. Each point is the average of a duplicate or triplicate determination. The lowest value reported is two standard deviations above the mean determination for the zero analyte control.

All data analysis and curve fitting was performed using GraphPad Prism.

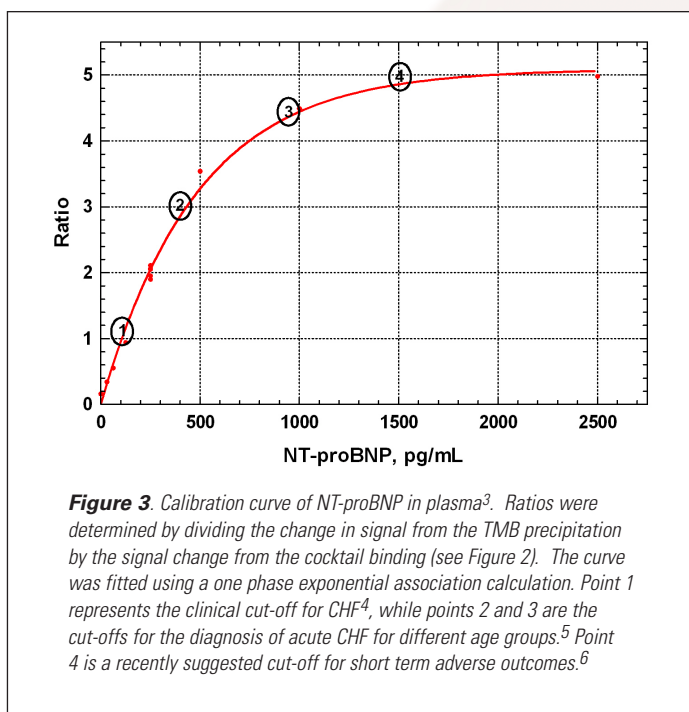
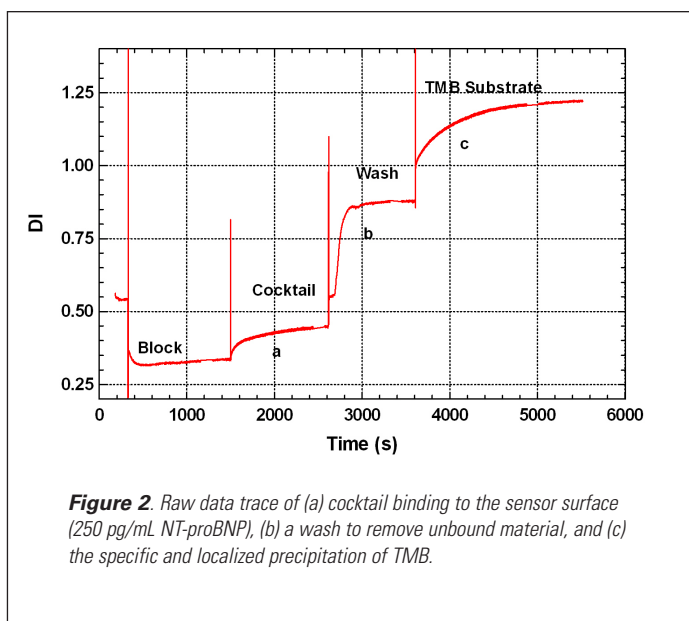
Conclusion

This indirect sandwich assay provides amplification of picomolar concentrations of protein. Calibration curves can subsequently be generated quickly for antibody or analyte quantitation from the resulting data. The sensitivity of the dotLab System provides a controlled method for screening complex biological samples.

References

¹Results generated using dotLab developmental systems and sensors, ²Axela Technical Brief 101, Diffractive Optics Technology, ³Clin. Chem., 2006; 52: 2168-70, ⁴Clin. Lab., 2005; 51(3-4): 167-72, ⁵Am. J. Cardiol., 2005; 95: 948-954, ⁶Eur. J. Heart Fail., 2005; 7: 575-565,

The dotLab System is for research use only. Not for use in diagnostic procedures.



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