

## Measurement of Endogenous Receptor Response on Suspension Cells

### Abstract

GPCR activation analysis is most often performed using recombinant cells over-expressing the GPCR of interest due to the sensitivity limitations of many screening assays. This requires researchers to take the additional step of creating recombinant cell lines or clonally selecting responsive cell lines. A less timely and more physiologically relevant system based on non-recombinant cells is highly desirable. An additional challenge for high throughput processing is the common need to use adherent cells. SRU Biosystems' BIND, label-free detection technology has the sensitivity required for detection of endogenous GPCR activation and is fully compatible with non-adherent, suspension cells eliminating the challenges faced by other detection chemistries. This application note describes the successful use a cell-based BIND assays for the label-free analysis of an endogenous G-protein coupled chemokine receptors in THP-1 suspension cells.

### Introduction

Chemokine receptors, a type of G protein-coupled receptor (GPCR), are involved in inflammation, cancer, and infectious diseases. Chemokine receptors have proven to be one of the more challenging GPCRs to adapt to some of the more commonly utilized screening technologies such as FLIPR. The current trend in pharmaceutical research to utilize more physiologically relevant assays earlier in the discovery process has driven the need for a high throughput technology that enables use of human cell lines expressing target receptor endogenously for lead profiling and screening. The use of a non-adherent cell line expressing endogenous chemokine receptor increases the challenge of establishing a robust assay for high throughput screening (HTS). BIND<sup>®</sup> not only provides a platform to develop an assay for any endogenous GPCR (Gi, Gq, Gs) but also reduces the number of cells per assay. Label-free BIND is a robust, sensitive technology for screening and profiling endogenous chemokine receptors in both adherent and suspension cell types, including primary cells.

The BIND<sup>®</sup> System provides a non-invasive assay method for analysis of receptor activation in live adherent and suspension cell types. The CA-1 BIND<sup>®</sup> Biosensor is utilized in this study, improving the response of suspension cells. BIND provides equivalent sensitivity when measuring either

recombinant or endogenous receptors. This study is focused on the endogenous GPCRs expressed in THP-1 cells, a non-adherent cell type. The potencies of chemokines on the native biological system are described.

### BIND<sup>®</sup> Technology

BIND from SRU Biosystems is a label-free universal assay system that enables detection of drug-target interactions. The system consists of the BIND<sup>®</sup> Reader and 96-, 384- and 1536-well microplate BIND Biosensors.

The BIND System takes advantage of a novel optical effect to provide very sensitive measurements of changes in binding

Figure 1

or adherence in the proximity of the biosensor surface. The biosensor incorporates a proprietary nanostructured optical grating, and is incorporated into microwell plates in industry standard formats. The grating of the BIND biosensor reflects only a single wavelength (“Peak Wavelength Value” or “PWV”).

When a cell binds to the biosensor surface, this reflected wavelength increases (Figure 1). Real time binding can be observed by measurement of the shift in PWV over time. For example, when cells are seeded onto the biosensor surface, cell attachment can be detected without the use of a label. Each addition of an assay component, such as a receptor ligand, to an assay well can be observed in real time to monitor the response of the cell.

## Materials and Methods

BIND® Reader (SRU Biosystems)

384-well CA-1 BIND® Biosensor, Part No. CA1-384-M (SRU Biosystems)

THP-1 cells, Cat # TIB-202 (ATCC) are grown and harvested according to the vendor recommended protocols. RPMI-1640 with 10%FBS and 1%Pen/Strep was used for growth of the cells and the starvation assay media was HBSS with 10mM HEPES, 2mM CaCl<sub>2</sub>, 2mM MgCl<sub>2</sub>, 5mM Glucose.

The ligands ATP (Sigma), UTP (Sigma), RANTES (Calbiochem), Macrophage Inflammatory Protein-1-alpha (MIP-1 $\alpha$ ) (Sigma) and Monocyte Chemotactic Protein 1 (MCP-1) (Sigma) are prepared in starvation assay media at the appropriate concentration for addition to the assay plate.

## BIND® Assay Step 1 (Optional)

### Starvation buffer only baseline

This step is helpful when developing assay conditions for cell density and seeding time. A 384-well CA-1 BIND Biosensor is removed from its packaging and 25 ul of cell assay media is dispensed into each well. Let the plate sit at room temperature for 10 minutes. A starvation assay buffer only baseline is obtained on the BIND Reader. Acquire baseline data for approximately 5 minutes.

## BIND® Assay Step 2

### Cell attachment signal

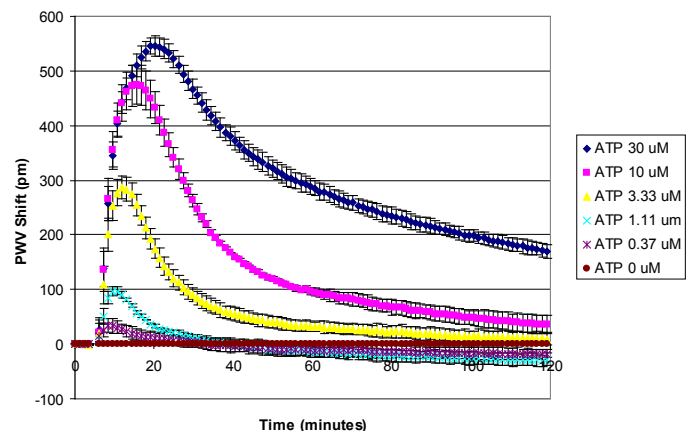
In screening mode, Step 2 would be the baseline step. Suspension cells are harvested by transferring them from the tissue culture flask to a 50 ml tube, spinning the cells for 5 minutes at 1000 rpm, aspirate media carefully so as not to disturb cell pellet, and resuspend in 10 ml of starvation assay media. Count the cells, and dilute in starvation assay media to the desired cell density. Add 25ul of cell suspension into

each well (the wells already contains 25 ul of starvation assay media) at densities ranging from 10,000 to 60,000 cells per well for optimal response. Note that cell number is dependent on the cell type. Incubate at 37°C for two hours and up to overnight. Let the plate sit at room temperature for 10 minutes before proceeding. Acquire data until a stable baseline is obtained (typically 5 minutes). In assay development mode baseline Step 2 to Step 1

## BIND® Assay Step 3

### Compound addition

Immediately after finishing BIND Assay Step 2, place the plate into an automated liquid handler and “stamp” compound(s) of choice (one compound per well in screening mode, or a compound serial dilution for cellular pharmacology assays). Acquire data for 20 to 30 minutes to record cell response to compound addition. The data acquired at this step should be baselined to the last data timepoint of the previous step. Export the data for analysis using EMS Export Wizard. The exported data can be analyzed with standard software or using the BIND® EC50 template. All data shown here were analyzed using the BIND EC50 template.

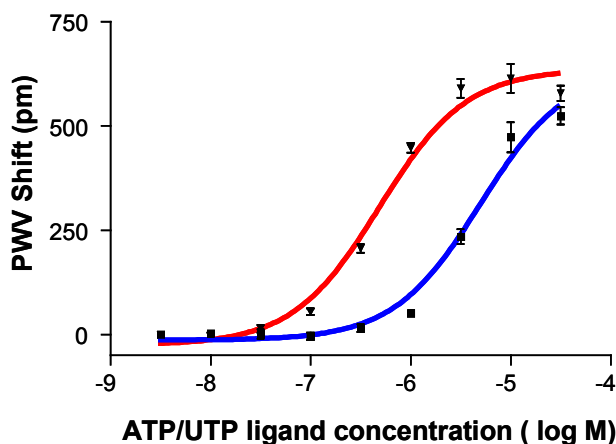


**Figure 2: Real time response of THP-1 cells expressing endogenous ATP receptor** THP-1 cells seeded on the 384-well CA-1 BIND Biosensor at 50K/well and treated with 30, 10, 3.3, 1.1, 0.37  $\mu$ M ATP. Cells treated with buffer alone (no ligand) were used as a background.

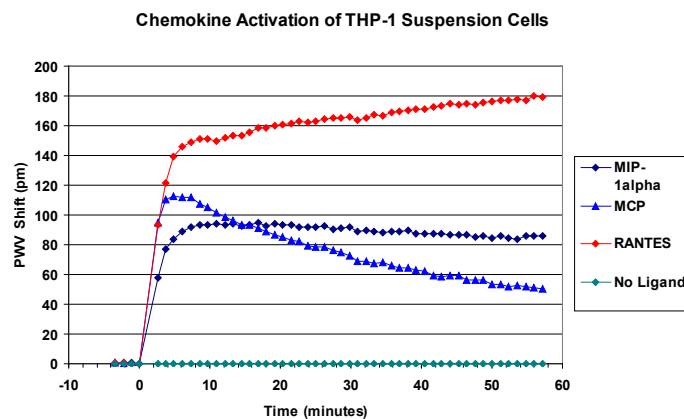
## Results and Discussion

Assay development and high throughput screening of cell-based assays using label-free BIND requires optimization of many of the same parameters which would also be used in other cell-based methods, such as cell numbers, seeding time and starvation time. Final DMSO concentration is always a consideration in screening assays, but it is important to maintain the same final DMSO concentration for each well to prevent bulk refractive index changes. It is recommended for dose response measurements that all concentrations are first diluted into DMSO followed by an equal volume addition to the final assay volume.

The CA-1 BIND Biosensor surface coating has been optimized to ensure that cells attach to the biosensor and provide a maximal response upon stimulation. The data in Figure 2 utilized 50,000 THP-1 cells per well. This provides a very robust signal of greater than 500 pm PWV shift with a very low background signal for unstimulated cells. The cell numbers can be reduced significantly if a lower cell response signal can be tolerated in the screen. The data analysis for EC<sub>50</sub> utilizes the maximum peak method. The EC<sub>50</sub>s obtained for ATP (4.9  $\mu$ M) and UTP (0.5  $\mu$ M) are consistent with published values (Figure 3). (Ref. 1,2) Robust response on THP-1 cells is also obtained with the chemokines MCP, MIP-1 $\alpha$  and RANTES (Figure 4).



**Figure 3: ATP/UTP Dose Response Measurement on THP-1 Suspension Cells** THP-1 cells seeded on the 384-well CA-1 BIND Biosensor at 50K/well and treated with 30, 10, 3.3, 1.1, 0.37  $\mu$ M ATP or UTP. Cells treated with buffer alone (no ligand) were used as a background. The EC<sub>50</sub> (ATP) = 4.9  $\mu$ M And EC<sub>50</sub> (UTP) = 0.5  $\mu$ M.



**Figure 4: Chemokine response in THP-1 suspension cells, using CA-1 BIND Biosensor 384-well microplates.** THP-1 cells were seeded into a 384-well BIND Biosensor coated with a proprietary suspension cell binding surface, CA-1. After 2 hours starvation, chemokine mediated response was monitored over time. All chemokines were used at 3  $\mu$ M concentration. Prior to injection of chemokine a stable baseline was obtained. Kinetic profiling of rapid cell response is possible with BIND. MCP maximum response peaks in 10 minutes, then rapidly declines. MIP-1 $\alpha$  and RANTES also reach maximum response rapidly but then remain stable over 60 minutes.

## Conclusions

- Suspension cells show a strong and robust response to chemokine ligands on the CA-1 BIND Biosensor.
- The system is optimal for compound screening in a high throughput mode, as well as for lower throughput compound lead profiling applications.
- The benefit of label-free cell-based assays is evident in the elimination of the cost of labeling reagents and the production of recombinant cell lines.
- The high sensitivity of label-free BIND allows measurement of endogenous receptor targets thus providing a more physiologically relevant assay for drug discovery.

## References

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14-A Gill Street  
Woburn, MA 01801  
Tel: 781.933.7255  
Fax: 781.933.5960  
Email: [info@srubiosystems.com](mailto:info@srubiosystems.com)

[www.srubiosystems.com](http://www.srubiosystems.com)

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