

# CORNING

The logo for Epic system, featuring the word "Epic" in a large, black, sans-serif font with a registered trademark symbol (®) to its upper right. The word "system" is written in a smaller, black, lowercase sans-serif font directly below "Epic". A thin vertical orange line is positioned to the left of the logo.

Epic<sup>®</sup>  
system

## **Study of Small-molecule/Protein Interactions Using the Corning<sup>®</sup> Epic<sup>®</sup> System**

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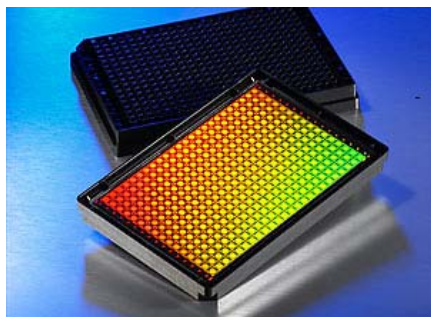
## Abstract

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We evaluated the Corning® Epic® System, a label-free, high-throughput detection technology, for detection and validation of interactions between small molecules and a nuclear receptor target. The nuclear receptor was directly immobilized via amine coupling in a 384-well Epic microplate. We first tested thirteen Novartis AG compounds against the nuclear receptor to determine yes/no binding and to estimate the binding affinity (KD) of two compounds identified as binders. We then tested these compounds as well as another set of Novartis AG compounds against streptavidin to identify/confirm compounds that bind in a non-specific manner. After getting satisfactory results in this initial study, we used quantitative HTS (qHTS) methodology to screen 690 small molecules, including true binders, known artifact compounds that cause false positive results, and inactive compounds (non-binders) against the nuclear receptor. The results presented here demonstrate that the Epic System is able to not only detect the direct interactions and rank relative binding affinities of small molecules against protein targets but also differentiate promiscuous aggregates from true binders. With relatively short assay development time--approximately two weeks--the Epic System provides a highly efficient and economic solution for secondary and primary drug screening.

# Corning® Epic® System

The Corning Epic System is a high-throughput, label-free detection platform that consists of SBS-standard 384-well microplates with optical sensors inside each well, an HTS-compatible microplate reader and a set of label-independent assay protocols. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of “intractable” targets.



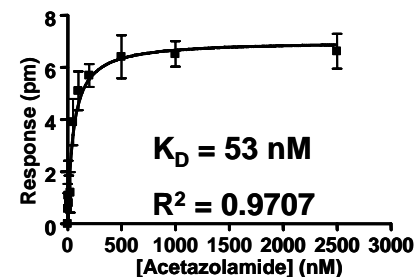
## Microplate

- 384-well format
- Optical biosensor in each well
- Surface chemistry



## Plate Reader

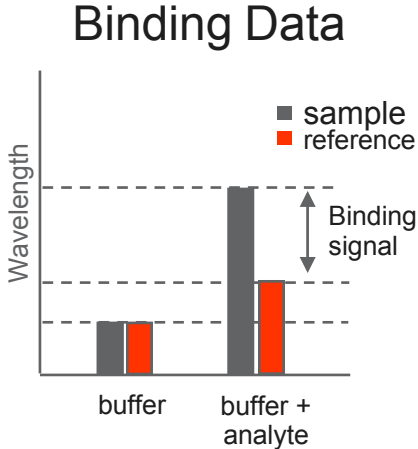
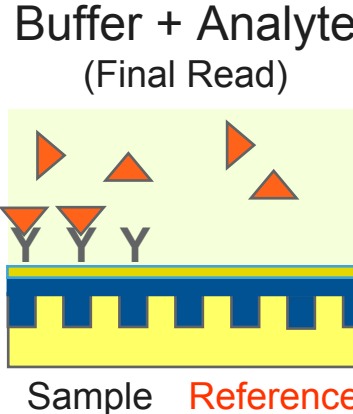
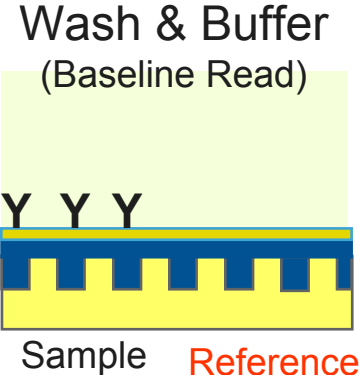
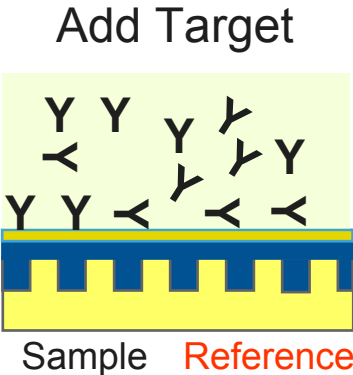
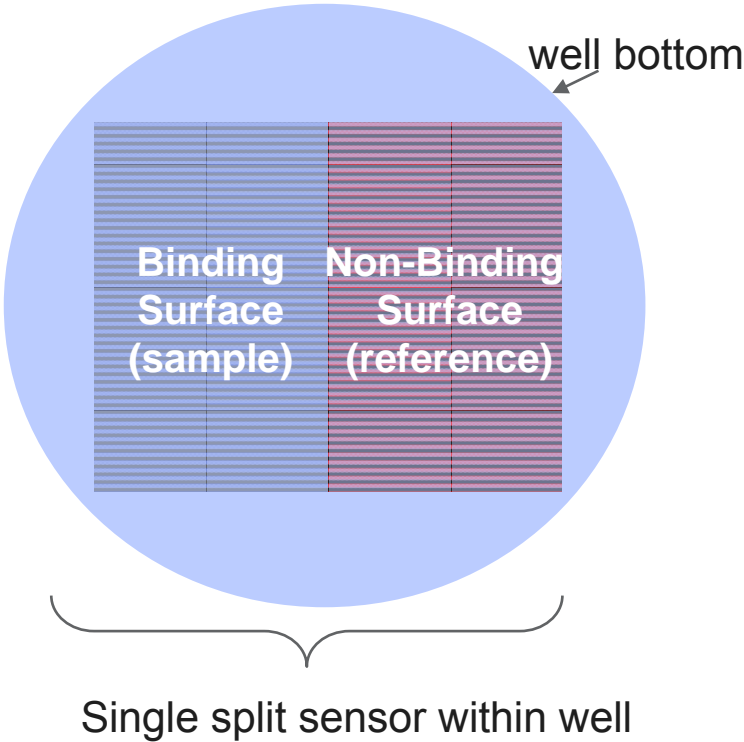
- Compatible w/ HTS automation
- $\geq 40,000$  wells/8hrs
- Sensitivity of  $5\text{pg}/\text{mm}^2$   
(300Da drug to 75kDa target)



## Binding Data

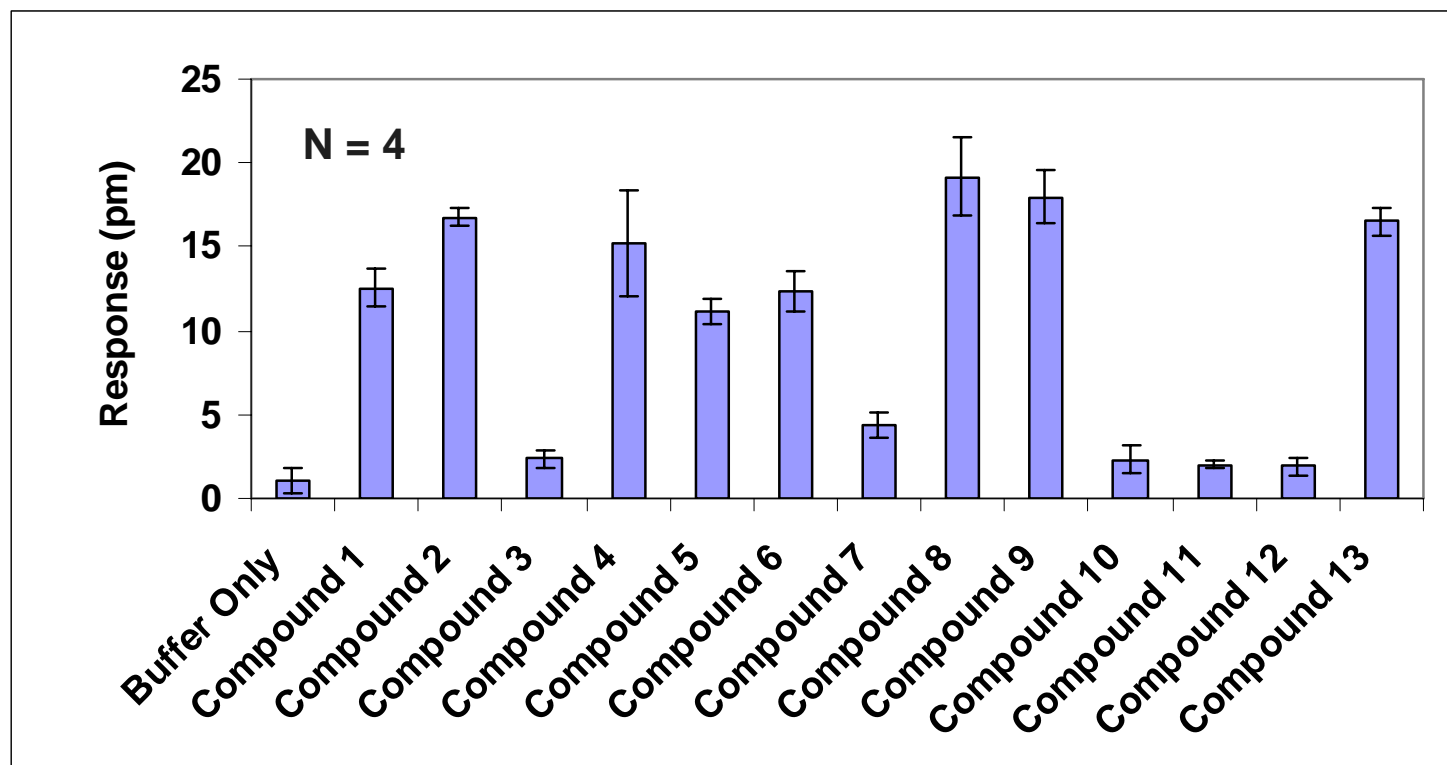
- Manipulated and analyzed by customer

# Dual-Sensor Self-Referencing Technology



## Mini-screen of 13 Compounds Against a Nuclear Receptor

- Nuclear receptor was immobilized at 50  $\mu\text{g/mL}$  in Na-Citrate pH 5/1mM DTT/0.02% CHAPS for 1 hour at room temperature
- Inhibitor (5 $\mu\text{M}$ ) binding assay performed in PBS with 0.1% DMSO/1mM DTT/0.02% CHAPS



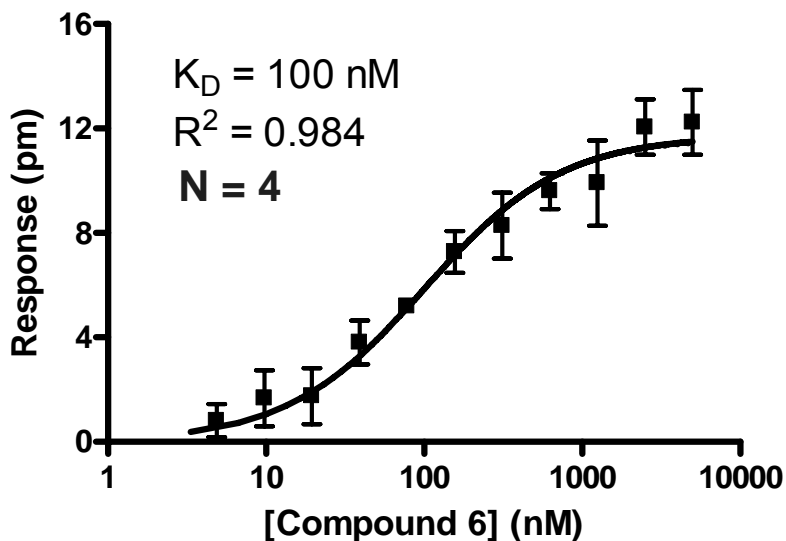
### Conclusions:

Eight compounds show binding to the nuclear receptor, consistent with Novartis internal data.

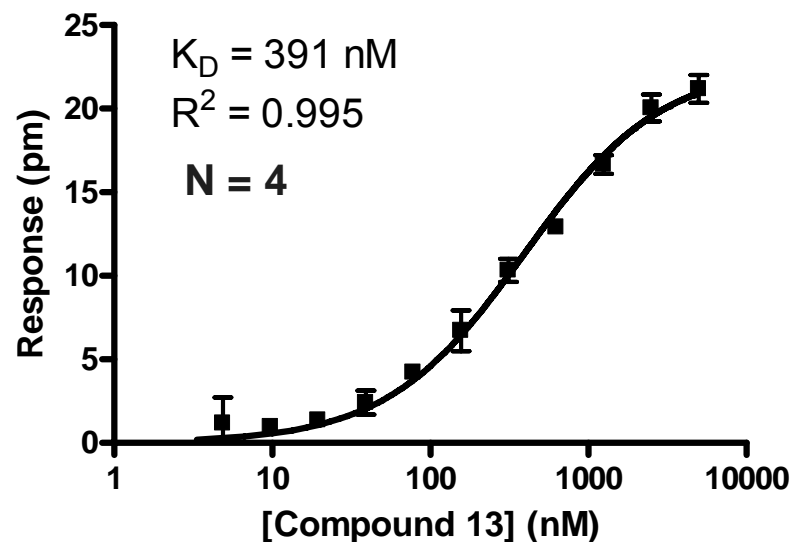
## $K_D$ Estimation of Compound 6 and 13 Binding to a Nuclear Receptor

- Nuclear receptor was immobilized at 50  $\mu\text{g/mL}$  in Na-Citrate pH 5/1mM DTT/0.02% CHAPS for 1 hour at room temperature
- Inhibitor titration performed in PBS with 0.1% DMSO/1mM DTT/0.02% CHAPS

TR-FRET:  $EC_{50} = 50 \text{ nM}$



TR-FRET:  $EC_{50} = 700 \text{ nM}$

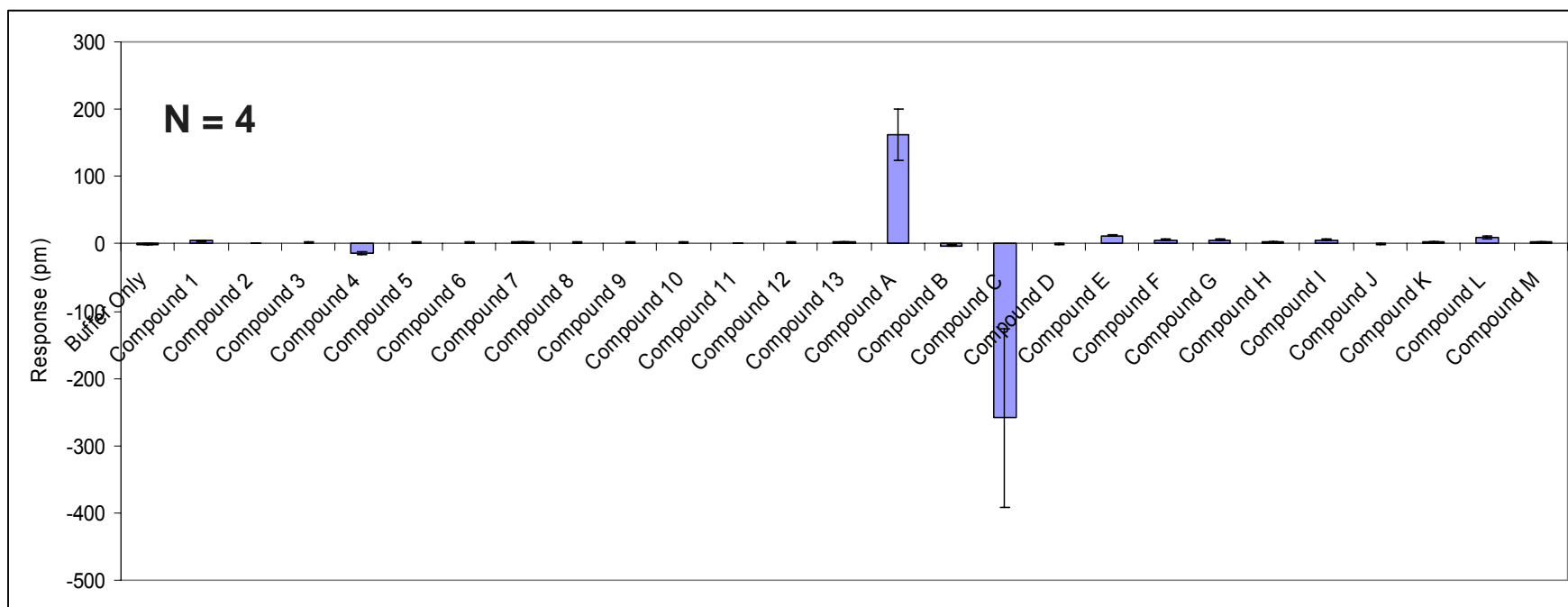


### Conclusions:

- Dose-dependent binding was observed for both Compound 6 (~160 Da) and 13 (~330 Da).
- $K_D$  estimations of both compounds were consistent with Novartis internal data.

## Mini-screen of 26 Compounds Against Streptavidin

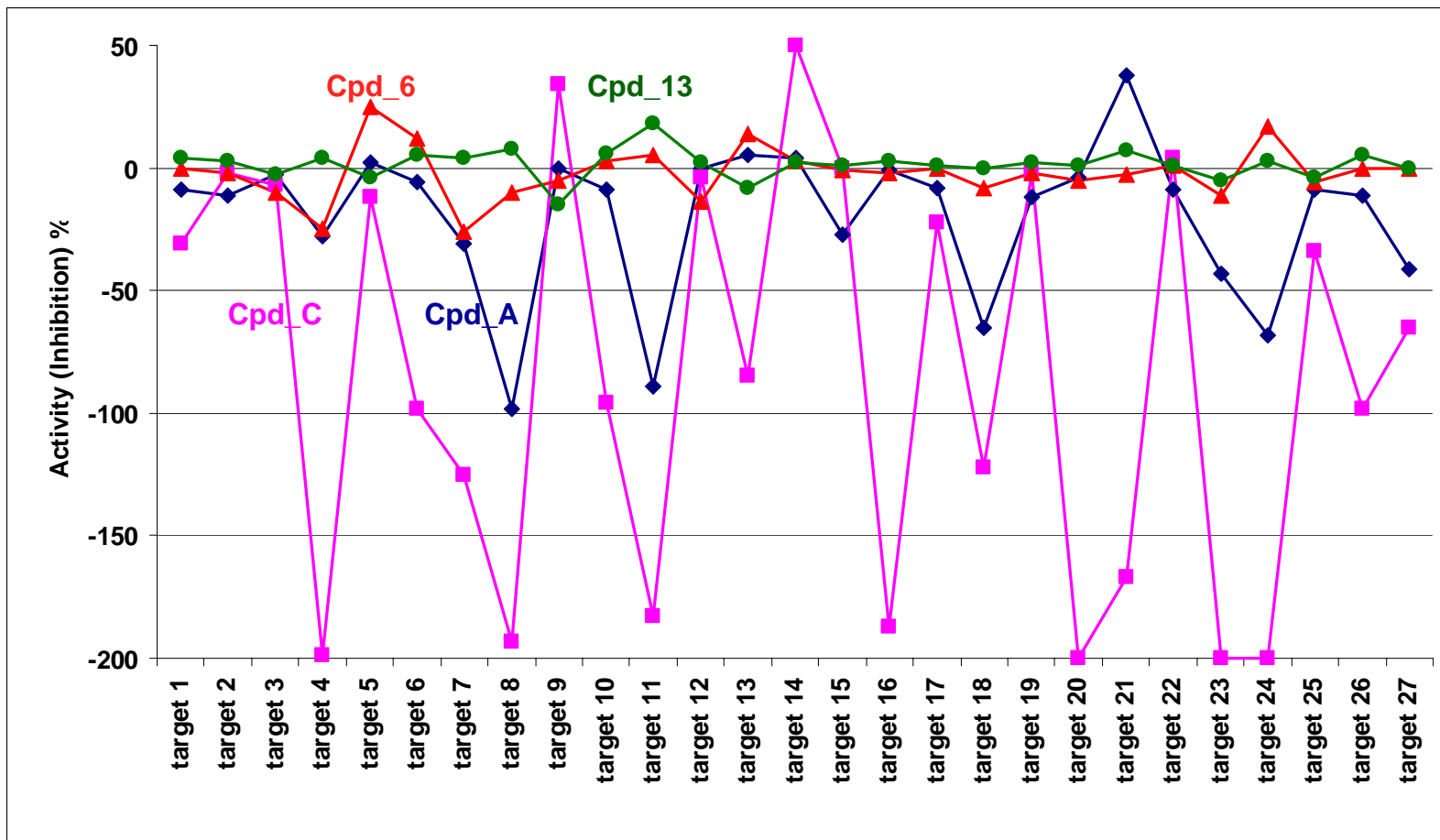
- Streptavidin (SA) was immobilized at 50  $\mu\text{g/mL}$  in 20 mM Na-Acetate pH 5.5 for 1 hour at room temperature
- Inhibitor (5 $\mu\text{M}$ ) binding assay performed in PBS with 0.1% DMSO/1 mM DTT/0.02% CHAPS



### Conclusions:

- Two promiscuous compounds (A & C) observed: abnormal binding signals observed for Compound A and Compound C, consistent with previous results.

# HTS Results of Four Compounds Against 27 Different Targets Based on Other Technologies



## Conclusions:

- Two promiscuous compounds (A & C) showed false positive results in a number of unrelated targets, consistent with Epic® results.

## Compound Set for Test

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- 690 compounds were tested in a dilution series of 8 concentrations (1:2 dilution) from 0.13uM-16.7uM.
- The compound test set contains
  - true binder, active agonists (187 compounds)
  - wrong active agonists detectable in counter screen (48 compounds)
  - true binder, active antagonists (41 compounds)
  - Known artifact compounds from HTS “challenge compounds” (155 compounds)
  - (86 compounds with false positive signals in target HTS)
  - Inactive non-binder compounds (95 compounds)
  - HTS hits not followed up so far (unknown binding) (164 compounds)
- From the data we wanted to be able to judge if the compounds are
  - target binders
  - non-binders
  - promiscuous/aggregating compounds

## Definitions for Compound Screen Data

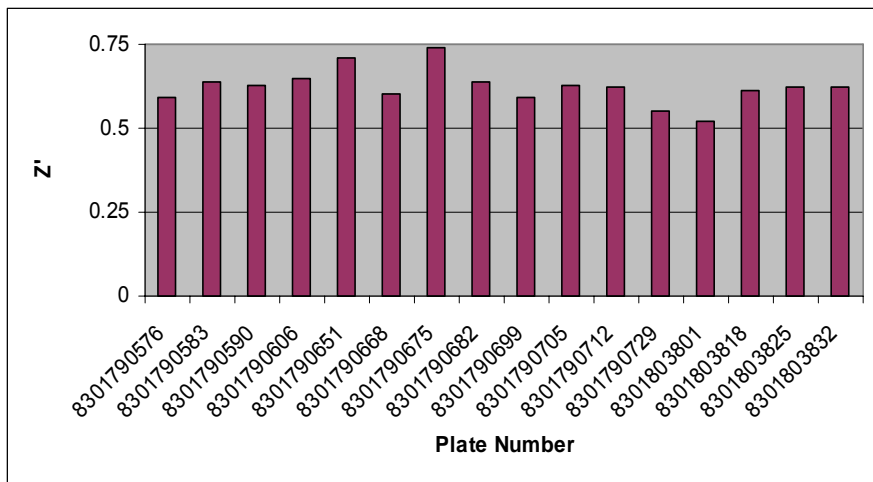
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The following are the general guidelines used in classifying the screen results:

- Clear Binder
  - Signal is within theoretical maximum
  - Signal is dose dependent
  - More than two concentrations show binding signal
- Questionable Binder
  - Signal is within theoretical max
  - Only 1-2 concentrations show binding signal
- Promiscuous/Aggregator (P/A compound)
  - Signal is larger than theoretical maximum OR signal is large and negative
  - Binding is observed to both target 1 and streptavidin
- Non-Binder
  - Signal is similar to buffer control
  - No dose dependency observed
- The theoretical maximal binding signal for a 500 Da compound to Target 1 is ~55 pm (1:1 binding)
  - $2500\text{pm (average immobilization signal)} \times (500\text{Da}/30\text{kDa}) \times 1.3$  (assumed ratio of refractive indices between Target 1 and a compound)

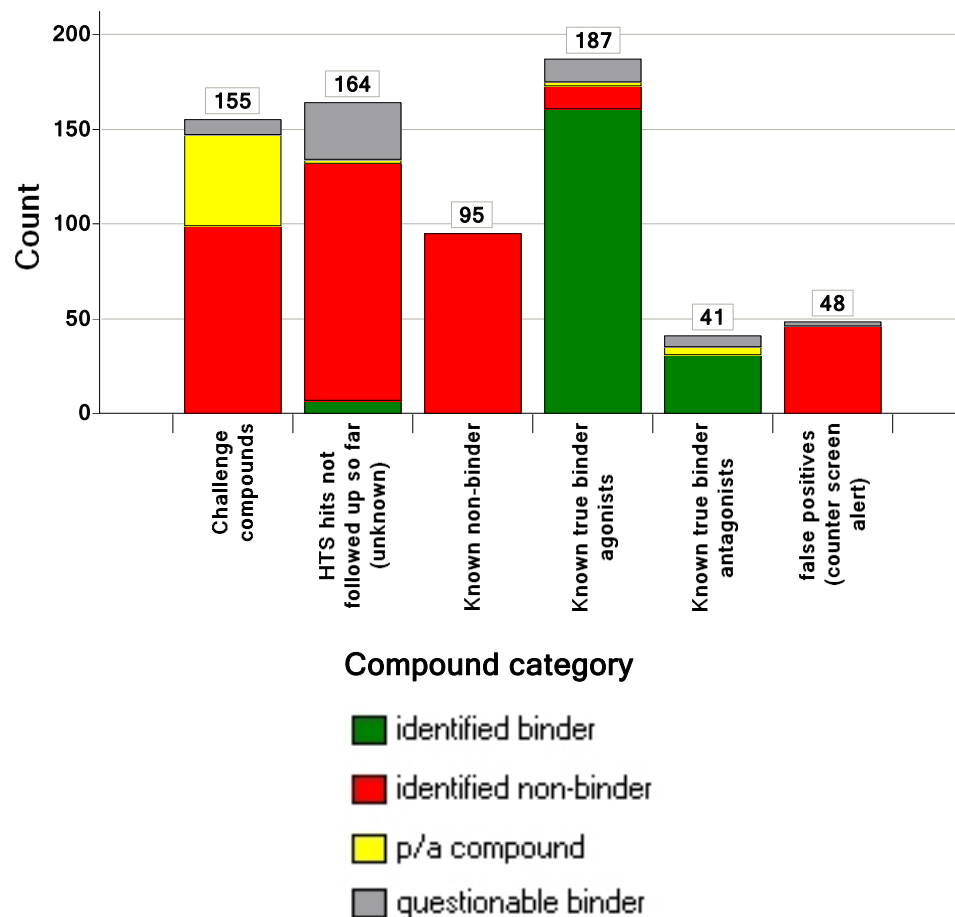
# Summary of Epic<sup>®</sup> Study Results

## Z' of Sixteen Plates (N = 8)



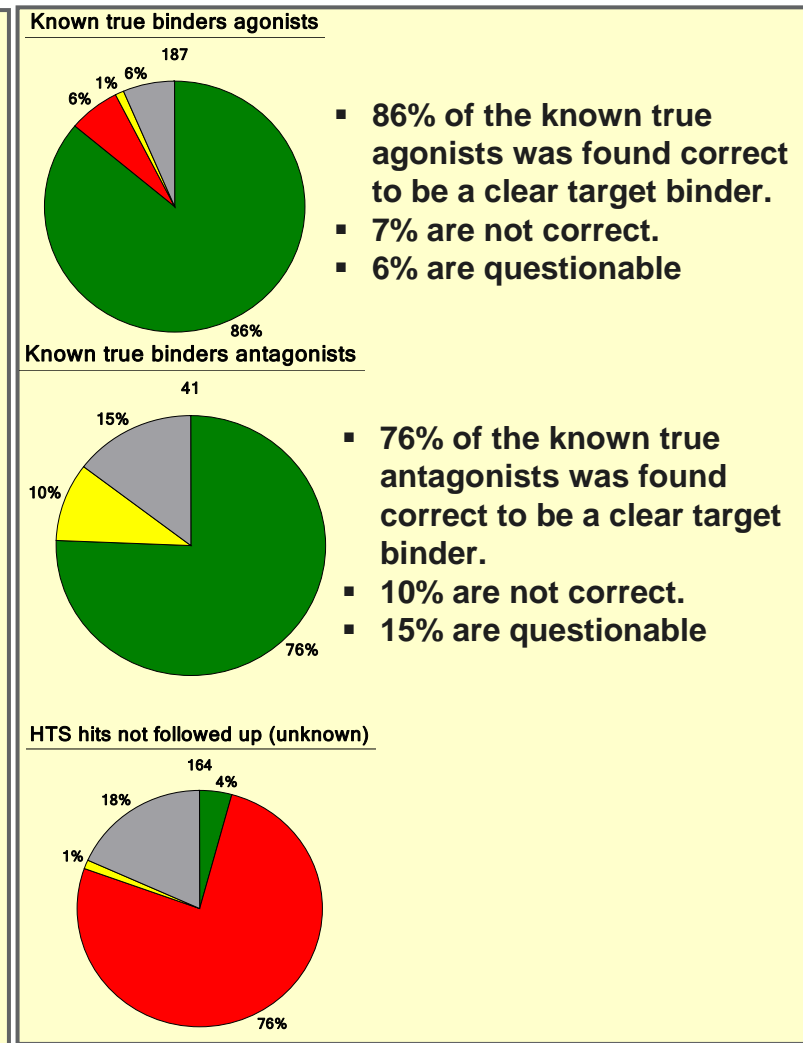
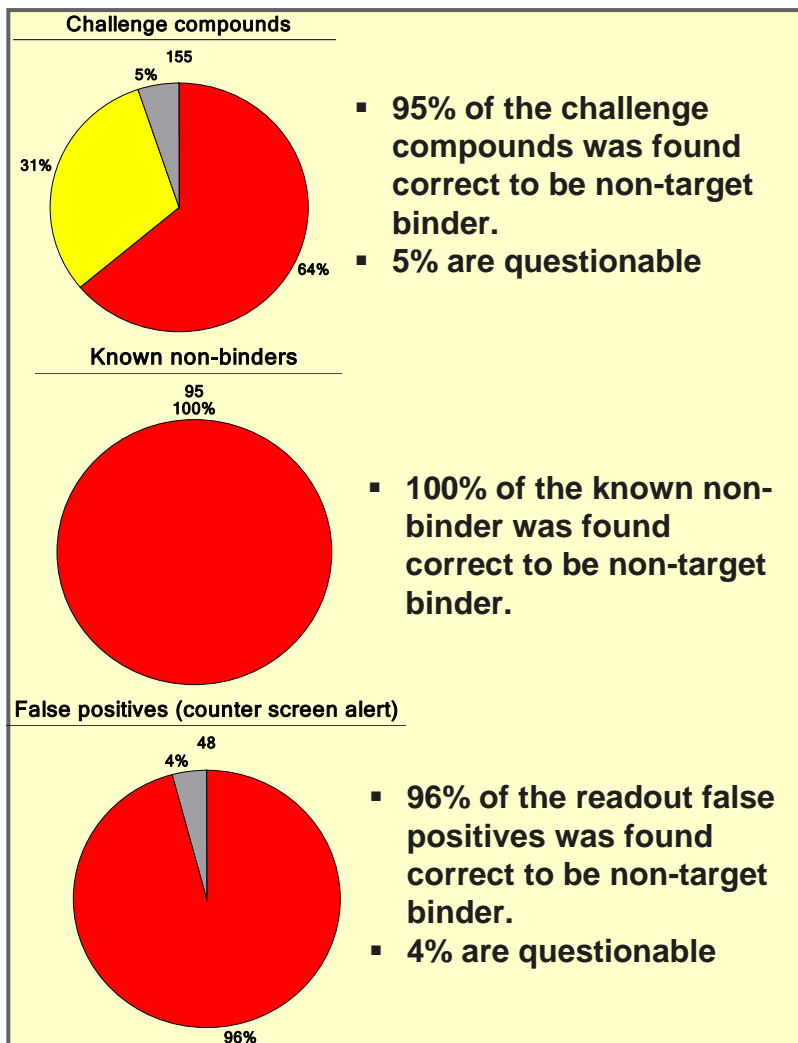
The performance of the Epic assay was good, all plates with a Z' > 0.5

## Epic Results Summary



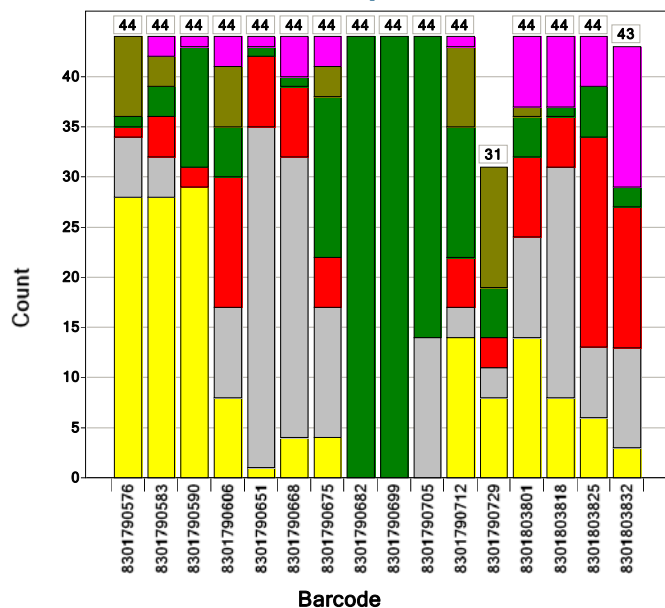
# Comparison of Epic<sup>®</sup> Results with Those from Other Technologies

- identified binder
- identified non-binder
- p/a compound
- questionable binder



# Microplate Results

## Distribution of compound classes over plates



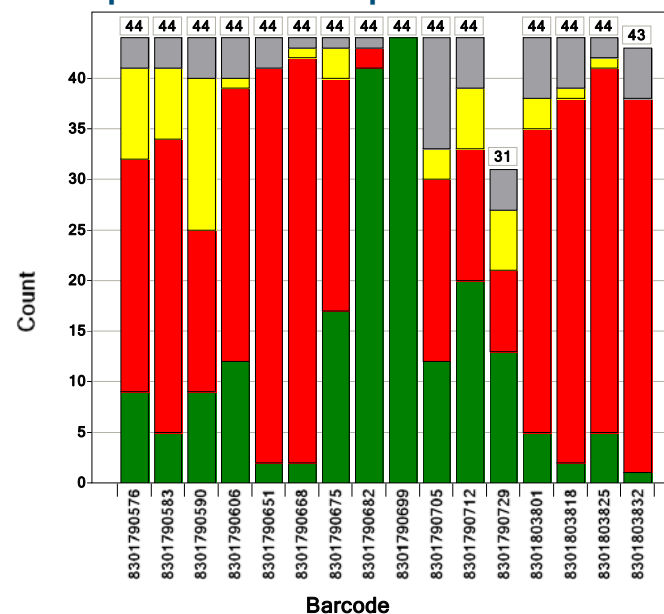
The height of a bar represents the number of records.

Color by Compound category:

- Challenge compounds
- HTS hits not followed up so far (unknown)
- Known non-binder
- Known true binder agonists
- Known true binder antagonists
- Not tested in EPIC system
- false positives (counter screen alert)

The labels show the height of each bar.

## Epic® results over plates



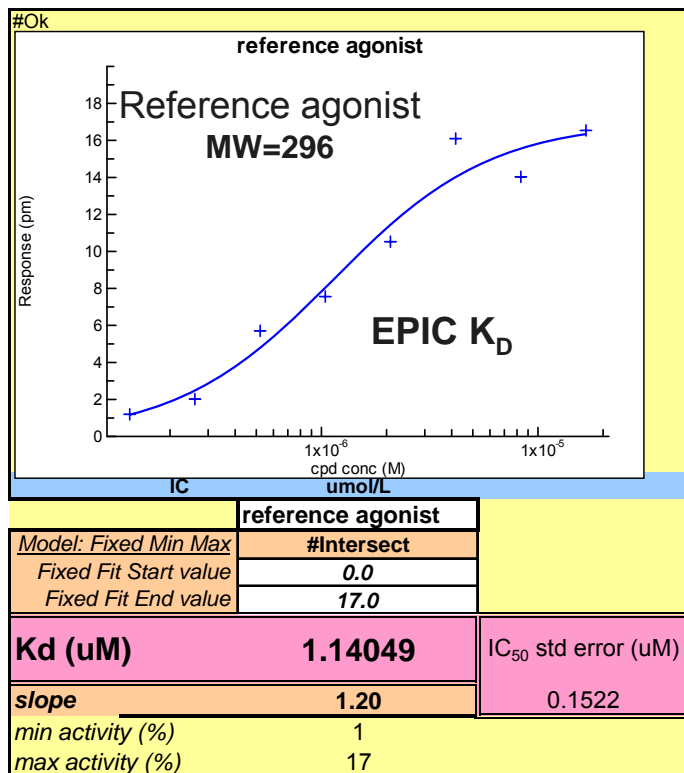
The height of a bar represents the number of records.

Color by Epic study judgement:

- Unknown
- identified binder
- identified non-binder
- p/a compound
- questionable binder

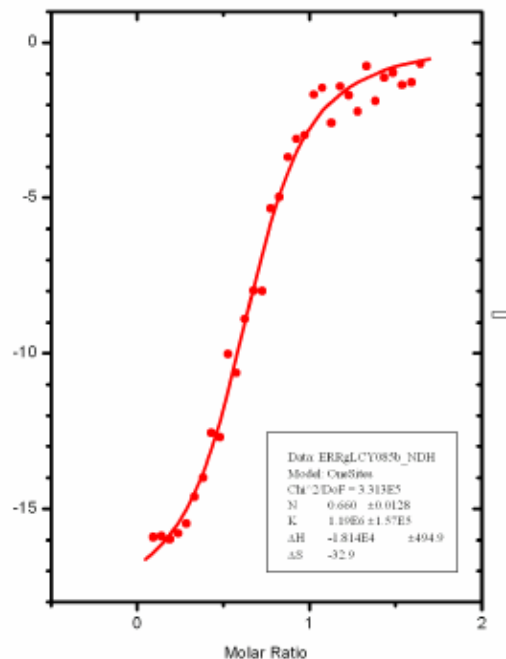
The labels show the height of each bar.

# Epic<sup>®</sup> K<sub>D</sub> and Data From Other Technologies

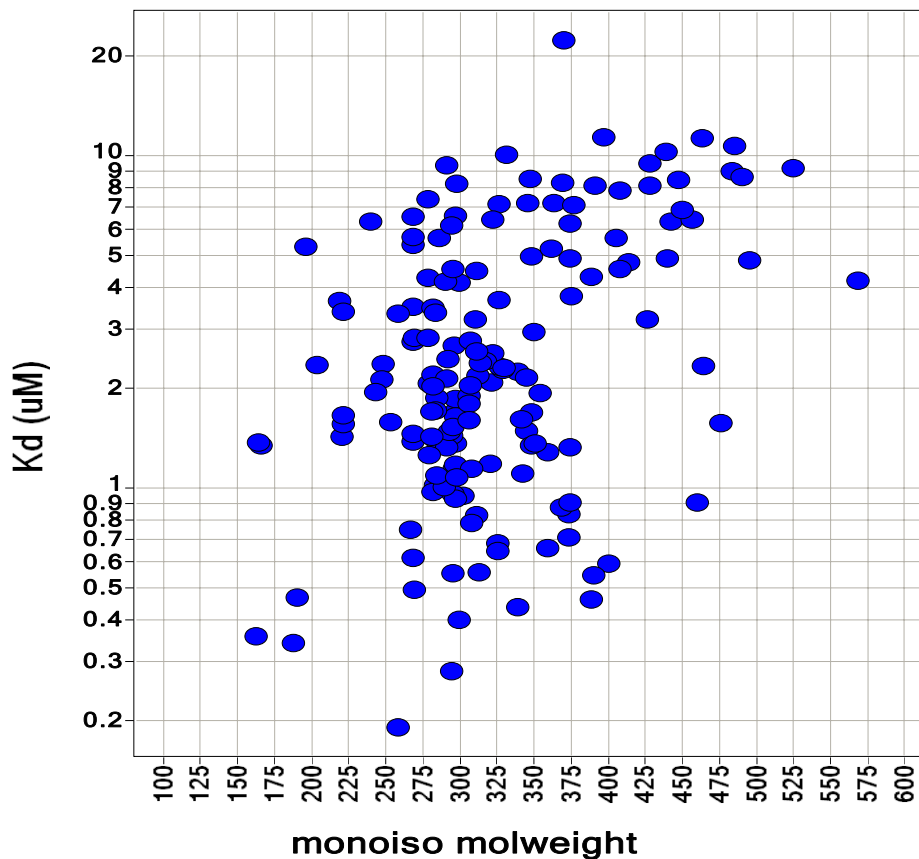


K<sub>D</sub> data:

- Epic: 1.14 µM
- Biacore: 3.2 µM ( $k_a = 1012$  1/M\*s;  $k_d = 0.0032$  1/s))
- ITC: 0.81 µM (stoichiometry = 1)



## Epic<sup>®</sup> K<sub>D</sub> versus MW

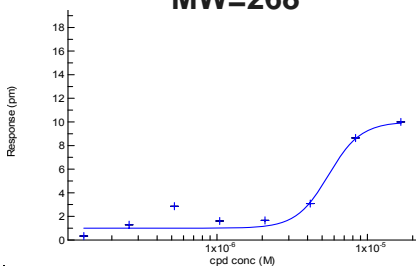


### Conclusions:

- The Epic System is able to detect the binding of molecules as small as 160Da with a range of affinities (nM to μM).

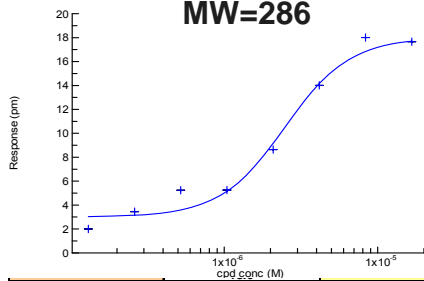
# Epic<sup>®</sup> K<sub>D</sub> Examples

HTS antagonist  
MW=268



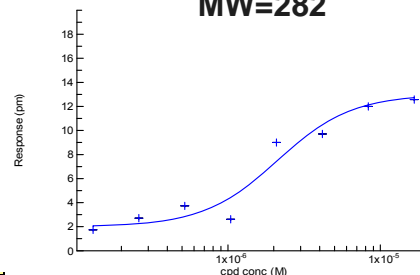
|                  |                |                                 |
|------------------|----------------|---------------------------------|
| <b>Kd (uM)</b>   | <b>5.51228</b> | IC <sub>50</sub> std error (uM) |
| <b>slope</b>     | <b>4.04</b>    | 0.5864                          |
| min activity (%) | 0              |                                 |
| max activity (%) | 10             |                                 |

HTS antagonist  
MW=286



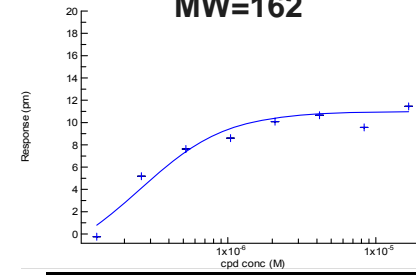
|                  |                |                            |
|------------------|----------------|----------------------------|
| <b>Kd (uM)</b>   | <b>2.48188</b> | IC <sub>50</sub> std error |
| <b>slope</b>     | <b>2.05</b>    | 0.2267                     |
| min activity (%) | 2              |                            |
| max activity (%) | 18             |                            |

Literature reference  
MW=282



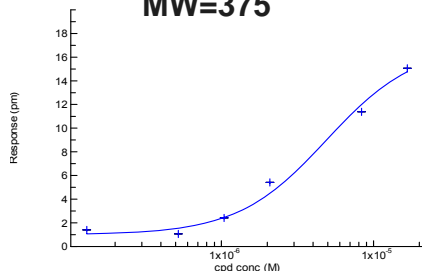
|                  |                |                                 |
|------------------|----------------|---------------------------------|
| <b>Kd (uM)</b>   | <b>2.11060</b> | IC <sub>50</sub> std error (uM) |
| <b>slope</b>     | <b>1.78</b>    | 0.3272                          |
| min activity (%) | 2              |                                 |
| max activity (%) | 13             |                                 |

HTS agonist  
MW=162



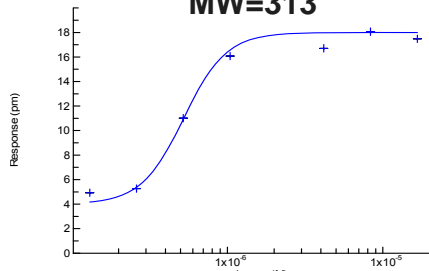
|                  |                |                                 |
|------------------|----------------|---------------------------------|
| <b>Kd (uM)</b>   | <b>0.25460</b> | IC <sub>50</sub> std error (uM) |
| <b>slope</b>     | <b>1.48</b>    | 0.0301                          |
| min activity (%) | 0              |                                 |
| max activity (%) | 11             |                                 |

HTS agonist  
MW=375



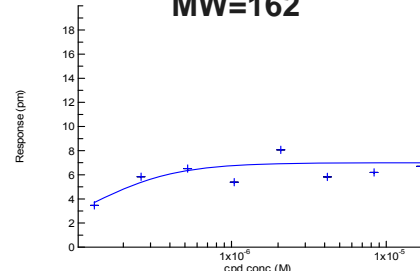
|                  |                |                                 |
|------------------|----------------|---------------------------------|
| <b>Kd (uM)</b>   | <b>4.90634</b> | IC <sub>50</sub> std error (uM) |
| <b>slope</b>     | <b>1.49</b>    | 1.0816                          |
| min activity (%) | -2             |                                 |
| max activity (%) | 15             |                                 |

Project agonist  
MW=313



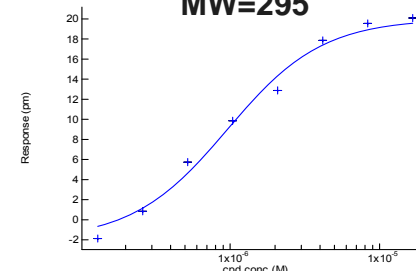
|                  |                |                                 |
|------------------|----------------|---------------------------------|
| <b>Kd (uM)</b>   | <b>0.52387</b> | IC <sub>50</sub> std error (uM) |
| <b>slope</b>     | <b>3.17</b>    | 0.0501                          |
| min activity (%) | 5              |                                 |
| max activity (%) | 20             |                                 |

HTS agonist  
MW=162



|                   |                |                                 |
|-------------------|----------------|---------------------------------|
| <b>Kd (uM)</b>    | <b>0.12104</b> | IC <sub>50</sub> std error (uM) |
| <b>Hill Coef.</b> | <b>1.56</b>    | 0.0440                          |
| min activity (%)  | 3              |                                 |
| max activity (%)  | 8              |                                 |

Project agonist  
MW=295



|                  |                |                                 |
|------------------|----------------|---------------------------------|
| <b>Kd (uM)</b>   | <b>0.96140</b> | IC <sub>50</sub> std error (uM) |
| <b>slope</b>     | <b>1.37</b>    | 0.0751                          |
| min activity (%) | -2             |                                 |
| max activity (%) | 20             |                                 |

## Conclusions

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- We correctly identified compounds that bind to the nuclear receptor;
- $K_D$  estimates gave proper affinity ranking of compounds;
- A unique feature of the Epic<sup>®</sup> System is its ability to identify promiscuous compounds and reduce false positives;
- We correctly identified those compounds known to be non-specific binders and/or promiscuous compounds;
- The screen results were more than 90% in agreement with Novartis internal data generated with other technologies.