Assay Development and High Throughput Screening Using Array Technologies and Electrochemiluminescence Detection

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Introduction

The Meso Scale Discovery™ (MSD) platform is based on the electrochemical properties of the ruthenium cation in conjunction with carbon electrode arrays held within microtitre plate footprints. In this poster we describe how we have used MSD technology together with CCD imaging using the Sector HTS™, to develop and screen robust assays in 384 well format to successfully identify inhibitors of protein protein interactions. More information on assay development is discussed in a separate poster at the conference (Ludbrook *et al.*)

Technology Evaluation (pre-HTS)

Prior to implementation, the MSD technology was evaluated for suitability to use in HTS using the target of interest. Table 1 shows the tests performed together with the success criteria for each test.

	Success Criteria
compound interference test	<0.5% compounds
solvent compatibility	Z' >0.5 up to 4%
reproducibility (pIC_{50} standards)	SD <0.3 plates/days
robustness testing (10k n=2)	median, assay stats, hit rate
simulation of HTS process	75 x 384 plates/day = 1FTE

Table 1

During the reproducibility testing, two standard inhibitor compounds were assayed with eight replicate curves per plate, on three plates per day over five days. The intra and inter plate variability of one of these standards is shown in Figure 1.

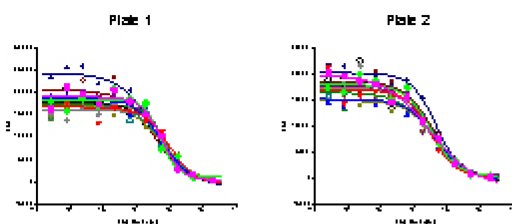

Figure 1

The final part of the technology evaluation was to screen approx. 10K random compounds in duplicate on two separate occasions under screening conditions. Figure 2 shows the correlation between the two replicates.

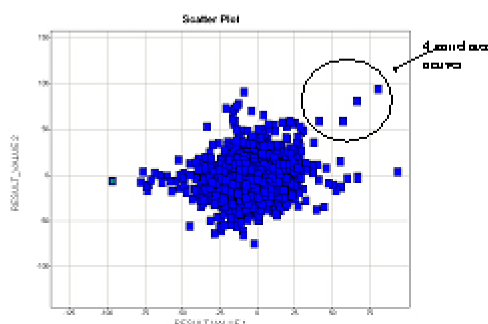

Figure 2

Assay Principle

The schematic below shows the assay principle (figure 3).

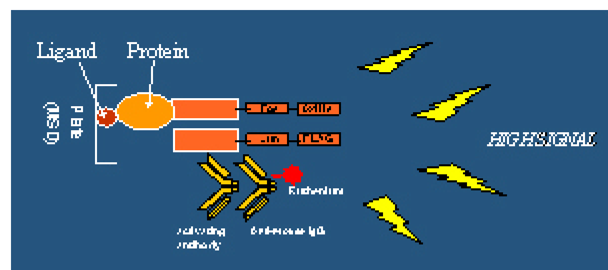

Figure 3

Assay Protocol

0.5µl 10ng/well Ligand passively immobilised onto MSD plate

↓

1µl 1mM compound, pre-diluted 1/20 with assay buffer (FAC 20µM)

↓

10µl diluted compound transferred to MSD plate

↓

15µl protein/activating Ab/Ru-IgG (1:2:4 ratio)

↓

Plates sealed, incubated for 5 hours @ RT

↓

10µl x4 Buffer T

↓

1 minute read on Sector HTS plate reader

Hardware for HTS

The screen was run manually (1FTE). A Cybio Cybiwell 384™ was used to transfer diluted compounds into the assay plate and all subsequent assay additions were performed using a Thermo Multidrop™. Following the final addition, the plates were read immediately on the MSD Sector HTS imager (Figure 4).

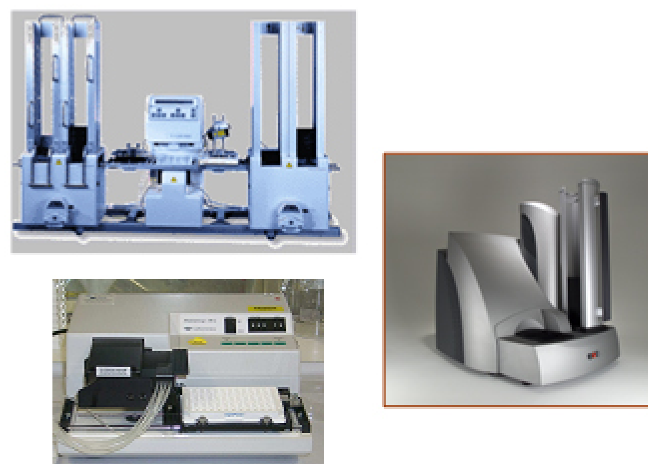

Figure 4

Screening Strategy

Ligand coated plates were prepared in advance in batches of approx. 200. The assay throughput was maintained at 75x384 well plates per day run as 3x25plate batches. Using this regime, the screen was completed in 27 working days.

Screen QC

As part of the daily run, a blank plate was included in each batch to identify any dispensing or reader errors. In addition, a QC plate was included. This plate contained a concentration response curve to a known standard inhibitor to track assay pharmacology throughout the course of the screen (Figure 5).

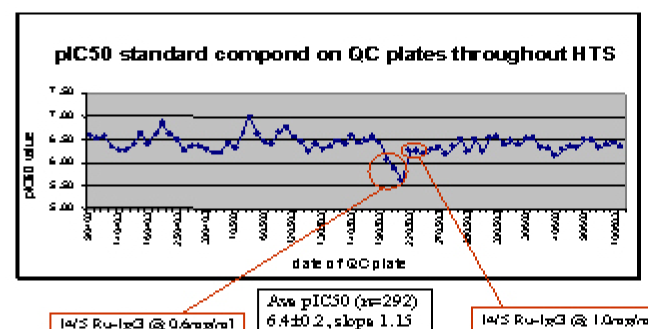

Figure 5

Assay performance is measured by calculating Z' value for each plate. Figure 6 shows the Z' values from all the compound plates run in the primary screening campaign.

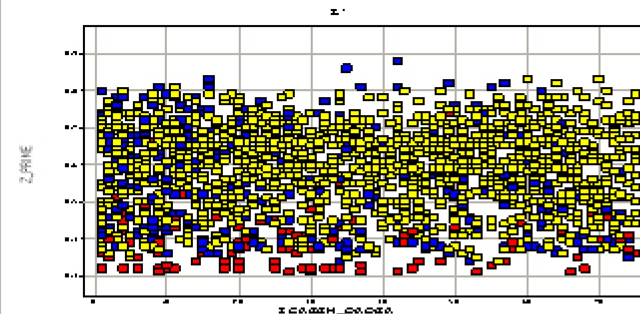

Figure 6

Primary Screen Statistics

Total number of compounds tested	532543	(501977 distinct OIs)
Total number of actives	17619	(17523)
Hit rate	3.3%	(3.5%)

Mean Z' value	0.6	(range 0.32 to 0.88)
Mean plate cut-off	30.0 %	(range 10.4 % to 47.4 %)

Total number plates screened	1971
Failure rate	
Biological	1.2%
Hardware	0.3%
Throughput 75 x 384 plates/day	

Compound Progression

Primary actives were subjected to chemical filtering to remove intractable compounds and then the data was clustered according to chemical class. A total of 2,568 compounds were selected for retesting. These compounds were assayed at the same concentration as the primary screen in duplicate. Figure 7 shows the correlation for the duplicates.

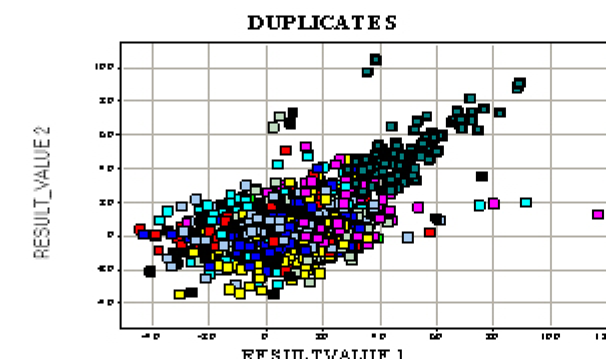

Figure 7

A total of 319 compounds were selected for IC_{50} determination. These compounds were prepared as 10pt concentration response curves using 1 in 2 dilutions. Compounds were screened in duplicate on two separate occasions. The pIC_{50} values for the replicates are shown in Figure 8. Following analysis, 14 of these compounds were shown to have activity against a closely related target.

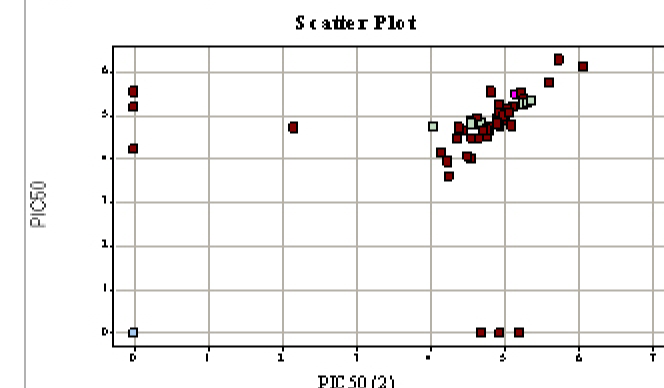

Figure 8

Conclusions

- For this target, MSD technology has proved enabling to completing a full diversity HTS.
- The screen quality was acceptable and several known inhibitors were identified in addition to new compound classes.
- The false positive rate was mainly due to high variability of ligand dispensing using passive adsorption.
- The assay was sensitive to evaporation meaning that sealing of plates was required during the screen which was not ideal.
- Compared to other technologies used within HTS at GSK, MSD was relatively expensive.

Reference

Ludbrook *et al.*, Poster P11022 9th SBS Conference Portland