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 coated with microporous plate technology. In this poster we describe how we have used MSD technology together with CCD imaging using the Sector HTS™, to develop and screen 24-well 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 this 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.

<table>
<thead>
<tr>
<th>Test</th>
<th>Success Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound selective test</td>
<td>&gt;20% compounds</td>
</tr>
<tr>
<td>Affinity (pIC50 of standards)</td>
<td>2&gt;IC50 &gt;4</td>
</tr>
<tr>
<td>Selectivity</td>
<td>10D &gt; 0.3 days</td>
</tr>
<tr>
<td>Nonspecific binding (hit rate)</td>
<td>0.0%</td>
</tr>
<tr>
<td>Simultaneous hits (percentage)</td>
<td>&gt; 1%</td>
</tr>
</tbody>
</table>

Table 1

During the reproducibility testing, two standard inhibitor compounds were assessed with eight replicate curves per plate, on three plates per donor over five days. The intra- and inter-plate variance of these standard is shown in Figure 1.

Figure 1

The final part of the technology evaluation was to screen approx. 10,000 compounds in duplicate on two separate occasions under screening conditions. Figure 2 shows the correlation between the two replicates.

Figure 2

Figure 3

Assay Principle

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

Figure 4

Assay Protocol

0.5μl 10μM stock Ligand passively immobilized onto MSD plate
1 μl 10μM compound, pre-diluted 1:100 with assay buffer (FAC 260μM)
10μl diluted compound transferred to MSD plate
15μl recombinating Ab1/Ab2 (1:24 ratio)
Plates sealed, incubated for 5 hours at RT
10 μl AB2 + Biotin T
1 minute read on Sector HTS plate reader

Hardware for HTS

The screen was run manually (ITFE), a CytoSoft CytoSoft 300™ 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 image (Figure 4).

Figure 5

Screen QC

Ligand coated plates were prepared in advance in batches of approx. 200. The assay throughput was maintained at 750-850 well plates per day run as 384-well batches. Using this regime, the screen was completed in 27 working days.

Screening Strategy

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

Figure 6

Figure 7

A total of 319 compounds were selected for KICp determination. These compounds were prepared as 1 μl concentration response curves using 1:2 dilutions. Compounds were screened in duplicate on two separate occasions. The pIC50 values from the replicates are shown in Figure 8. Following analysis, 14 of these compounds showed activity against a closely-related target.

Figure 8

Figure 9

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 dispenses making the whole screen negative.
• 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 6th SBG Conference Portland