A 1536 Well Epic® Assay Development Process

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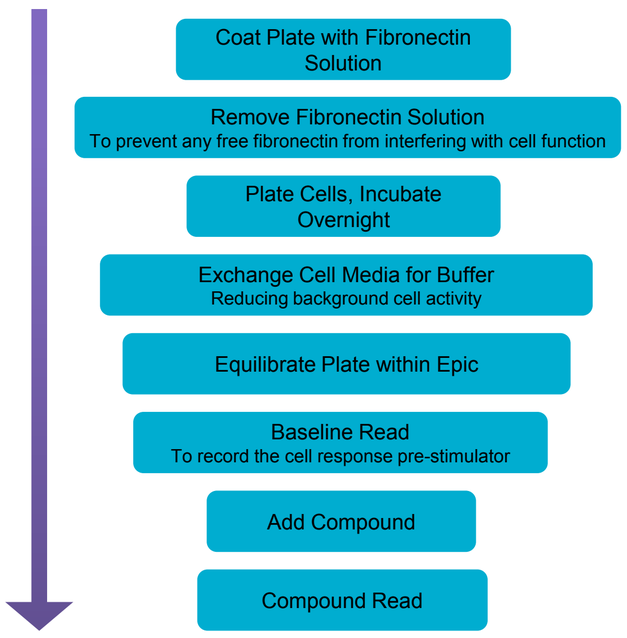
Corning Epic ® Technology - What's the Benefit?

The Corning Epic is a label free microplate reader able to measure the response of a cell to a drug via change in dynamic mass redistribution (DMR). DMR is measured by the refraction of light from a biosensor. Specialist microplates fitted with a glass biosensor at the well bottom are required but each of these plates costs several times more than a standard microplate. Often in lead discovery, the potential of pioneering technology such as the Epic is hindered by its cost. The High Throughput Screening department aim to maximise the number of compounds that can be screened against a target but at reduced cost. We describe the development of a 1536 format assay, to quadruple the number of compounds tested per plate and significantly reduce screening costs.

Figure 1 – Overview of the Epic Assay Sequence

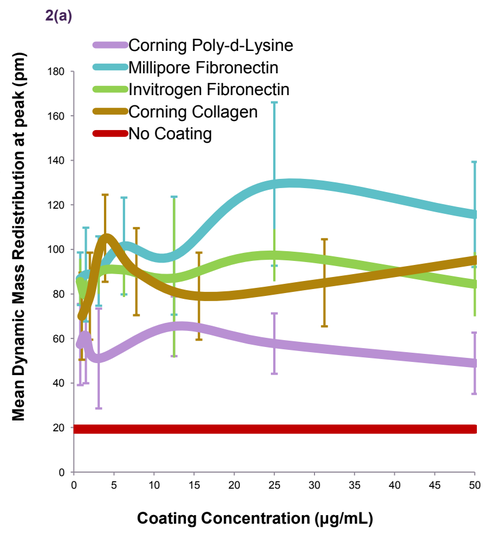


Miniaturisation Challenges – Fighting the Physics

The transition from 384 to 1536 well format was not as simple as expected. The low volumes are prone to evaporation and bubbles can persist due to the high surface tension in a small well. Intensive optimisation of liquid handling was required.
Additionally, the 1536 well plates required preparation using various protein solutions to permit cell adherence. Coatings recommended for GPCR-expressing cells were tested, as a CHO cell line over-expressing an orphan GPCR was used throughout optimisation.

Figure 2 – Plate Coating for Cell Adherence

2(a) Relative success of coatings, by peak response to a non-specific agonist (Zn^{2+}). Large error bars as experiment was pre-assay optimisation
2(b) Cell morphology on the coating which permitted the highest DMR, at 40x magnification. Coating with Fibronectin (Millipore) is comparable to commercially available 384 plates



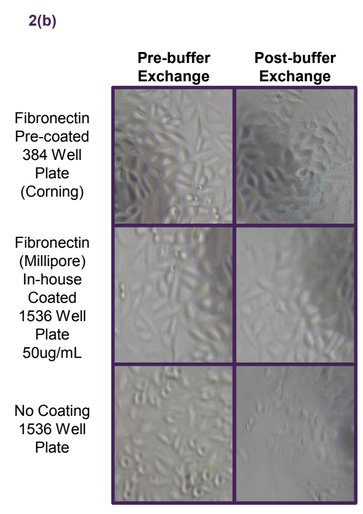
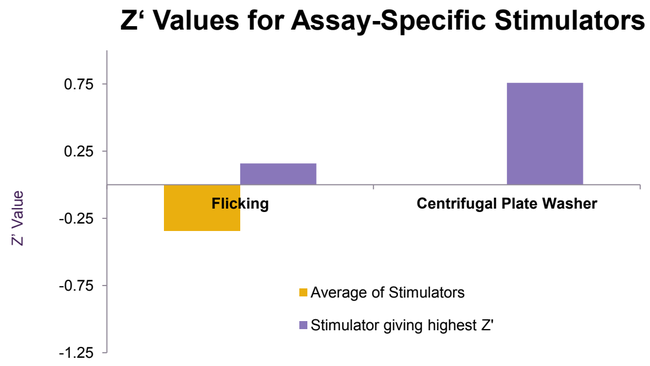


Figure 3 – Fibronectin Removal from a 1536 Plate

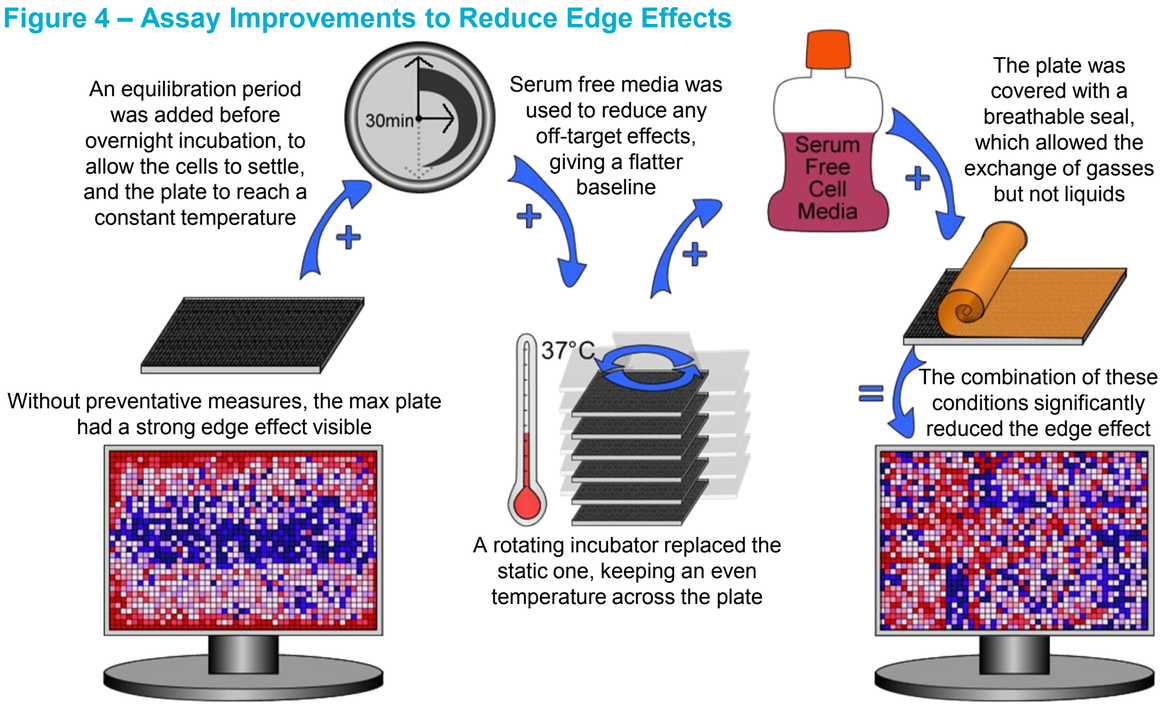
Comparison of Z' prime values, showing that removal of fibronectin using a centrifugal plate washer introduces the least variability into the assay. Stimulators gave a measure of cell viability, but did not specifically interact with the GPCR being over-expressed.



Evaporation and Edge Effects

Evaporation is a problem in 1536 well plates, as a small loss of liquid changes the concentration of reagents significantly. The plate edges are more susceptible to environmental temperature changes as they are less thermally shielded than central wells. This can produce an 'edge effect' in the plate signal.

Figure 4 – Assay Improvements to Reduce Edge Effects



Optimising the Liquid Handling

Initially reagents were added to the 1536 format assay using a Multidrop™ Combi, both for buffer exchange and compound addition. However the resultant plate pattern evident on a max signal plate showed that the Multidrop introduced variability into the assay.

Alternatives for Compound Addition

- FLIPR Tetra liquid handling - direct tip transfer between 1536 well plates
- Ideal, but was unavailable due to other screening commitments
- Vprep - dedicated pipetting automation
- Like FLIPR, but 384 format
- 4 separate transfers required, thorough washes between each. Introduces variability through compound carryover
- Echo - acoustic dispensing
- Non-contact transfer directly between 2 plates, eliminates carryover
- Requires compounds in 70-100% DMSO, which increases DMSO concentration in the assay. This changes the refractive index of the well and induces a non-biological increase in DMR.

Figure 5 – FLIPR Compound Transfer

Figure 6 – Effect of DMSO Mismatch on DMR

VPrep – Slight peak on addition of buffer control, due to disturbance of cells

Echo – Large peak on addition of buffer control due to DMSO mismatch. Negative DMR partially masked by it

Consequently, the VPrep was chosen

Buffer Exchange Options

As with compound addition, buffer exchange was initially a source of plate variation due to the use of a Multidrop. Improvements were seen in the %CV after replacement with a VPrep.

Figure 7 – Buffer Exchange with an Auswasher and Multidrop Combi

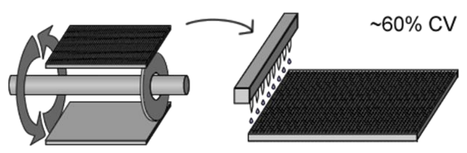
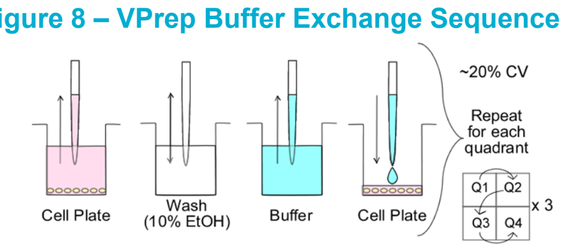


Figure 8 – VPrep Buffer Exchange Sequence



The Optimum Epic Read

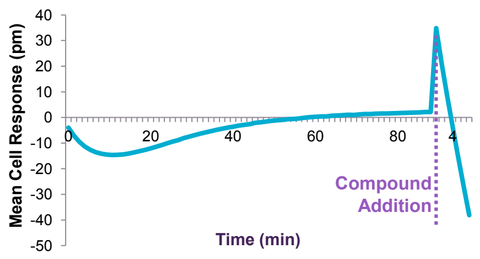
In order to screen the Epic assay at a scale suitable for high throughput screening the duration of both the baseline and compound reads had to be reduced as much as possible, to maximise the number of plates read per day.

Baseline Read

This gives a baseline reference for comparison with the compound response and should be flat when the cells are at equilibrium. 4 consecutive reads were sufficient for this.

Figure 9 – Kinetic Baseline Read

A max plate was read kinetically immediately after buffer exchange to assess the minimum equilibration time necessary. At 60min it is seen to level out.

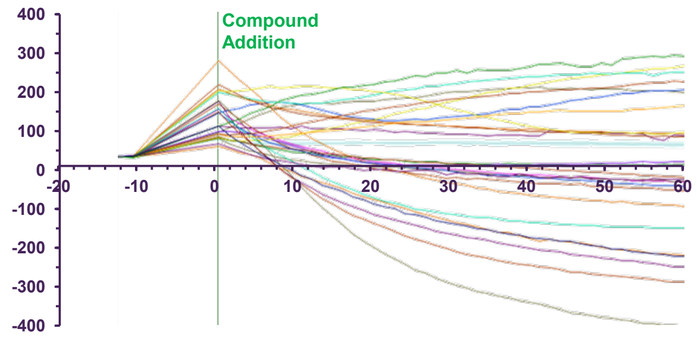


Compound Read

Determining when to take the compound read requires compromise as the peak response time is compound specific.

Figure 10 – Compound Read Determination

Kinetic responses of 26 compounds suggests that most are reaching a plateau by 20min



Concordance between 1536 Plates

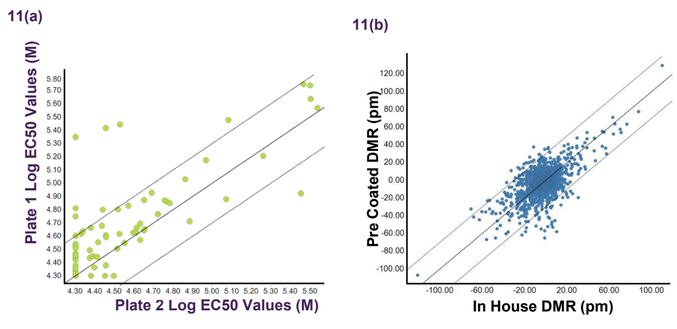
Once optimised, the correlation between assay plates was assessed.

Figure 11 – Comparison of 1536 plates

11(a) Log EC_{50} values of compounds tested on two identical 1536 format plates, coated in house with fibronectin. Curve fits show y=x±0.3 log

11(b) DMR of compounds at tested single concentration against cells plated on 1536 format fibronectin coated plates, one coated in house and one purchased commercially. Curve fits show y=x±30 pm

The majority of points fall within the curve fits showing good correlation between plates. The in house coating is comparable to the commercially available equivalent



Success of Assay Development

- In house fibronectin coating was comparable to the commercial equivalent
- Edge effects were successfully removed from the assay
- Plate read times were reduced for screening to increase throughput
- The VPrep was the best option available for liquid handling, although the assay would benefit from a dedicated 1536 platform
- Comparison to the 384 assay is required

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