



IncuCyte™ Applications

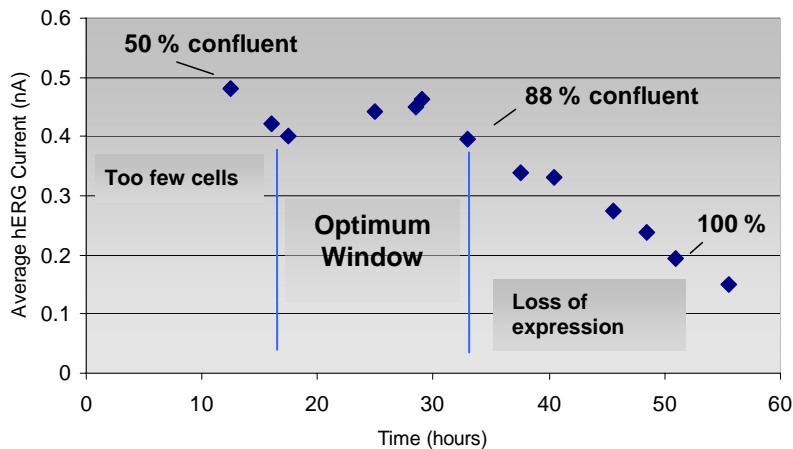
Cell-based Assay Optimization

Consistent and well optimized cell culture is a critical component in maximizing the day-to-day performance of a cell-based assay. The IncuCyte™ is a new tool ideally suited to the assessment and establishment of such optimal cell culture conditions.

Scientists at Essen have used this system to optimize the functional expression of hERG potassium current (host: CHO cells) for our high-throughput compound profiling assay in the IonWorks™ HT system.^[1,2] Because IncuCyte™ provides a non-invasive metric of proliferation, the same flasks which are monitored for confluence can subsequently be used to run in the IonWorks™ HT.

We have demonstrated that there is an optimum confluence “window” for the maintenance of hERG current activity, and that maintaining the cells within that window is necessary for optimum and consistent assay performance. Additionally, we have shown that if the parent flask reaches an overgrown state, cellular expression does not recover. Therefore, tracking of the parent flasks critical to the long-term stability of the assay. ***IncuCyte™ can be used to define an optimum assay window and help to insure that all cells (both for passaging and for assaying) are maintained within that window on a day-to-day basis.***

Optimization of hERG Assay



The chart to the left plots the mean hERG current (10^{-9} Amps from approximately 300 cells as measured in the IonWorks™ HT system) vs. time in culture. Horizontal axis is time (hours), starting from when the individual flasks achieved 30% confluence (as quantified by IncuCyte™).

An optimum time/confluence window exists for the assay based on the down-regulation of channel current concomitant with greater cell confluence. Having too few cells compromises the overall success rate of the assay, although channel expression is not affected.

Optimization Sequence:

1. Use IncuCyte™ to determine cell confluence.
2. Assay hERG channel activity with the IonWorks™ HT.
3. Map hERG activity vs. confluence to determine optimum assay window.
4. Monitor both assay and parental flask confluence with IncuCyte™ to maintain cells within appropriate confluence window.

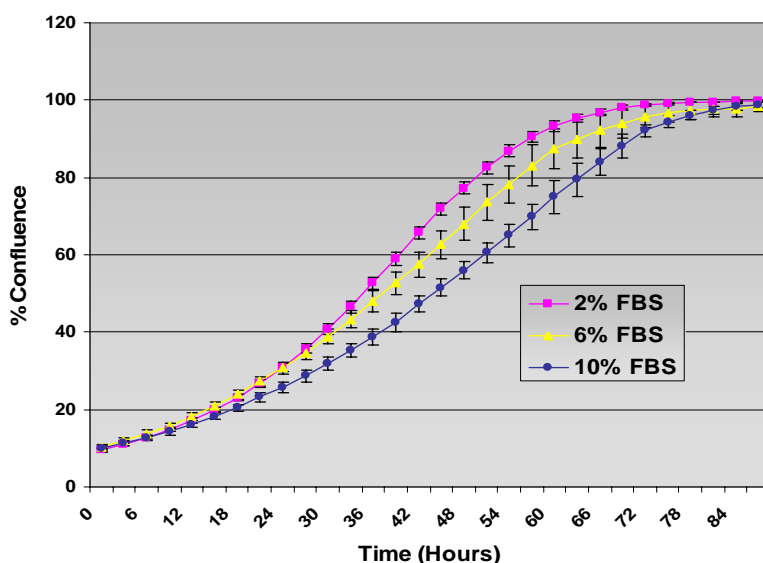
Cell Culture Media Optimization

Given the fast-paced reality of modern life science research, it is often impractical to consider optimizing cell culture conditions for each and every assay. Yet there is nothing more important to a cell-based assay than the cells themselves. IncuCyte™ can be used as a convenient, sensitive, and precise method to determine optimum growth conditions, including effects due to cell passage, media, serum, substrate, or environment. Furthermore, because IncuCyte™ provides “kinetic” proliferation curves and imagery, very subtle changes can be easily detected.

As an example, we have used this system to measure the effects of serum concentration on the proliferation of our CHO-hERG cell line. The graph below demonstrates that this cell line actually grows faster in the presence of 2% serum vs. the traditional 10% serum concentration.

Information such as this can be used to improve assay performance as well as to greatly reduce the costs associated with unnecessary cell culture supplements. The financial savings can be significant, in the tens of thousands of dollars, for an HTS campaign.

Serum Concentration vs. Growth Rate (CHO-hERG)



IncuCyte™ data displaying mean proliferation curves of CHO-hERG cells as a function of serum concentration. As indicated, these cells proliferate “faster” at 2% serum vs. 10% serum. Error bars are computed from n=12 wells.

^[1] Kiss, L., Schroeder K., “High Throughput Ion-Channel Pharmacology: Planar Array Based Voltage Clamp.” *Assay and Drug Development*, Vol 1, No 1-2, 2003.

^[2] Schroeder, K., Worley J., “ IonWorks™ HT: A New High Throughput Electrophysiology Platform.” *Journal of Biomolecular Screening*, Vol.8 (1), 2003.