Scintillant BIOSCIENCE

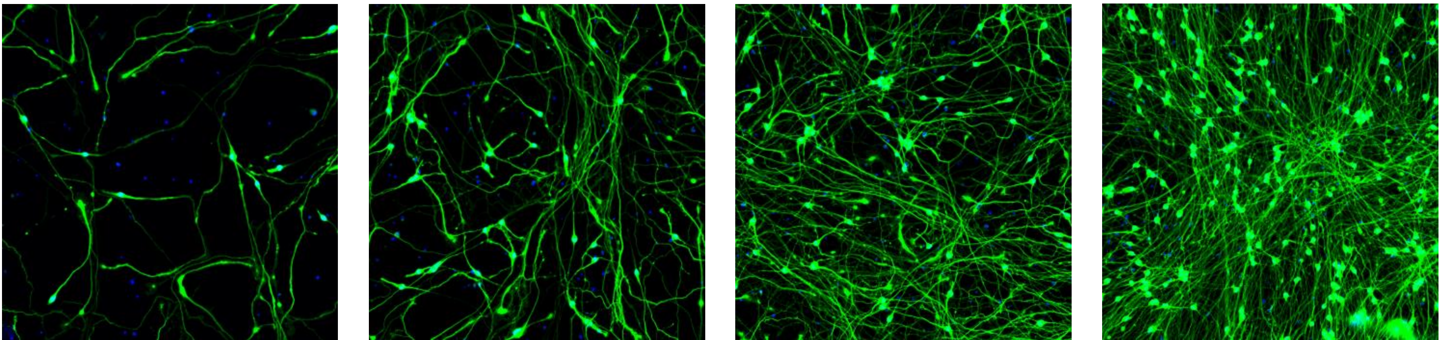
Tips and Tricks for Cell-Based Imaging Assays: Part 1

We are pleased to introduce the fourth installment in our ongoing series of white papers. In our previous three papers, we explored a range of topics, including theoretical and practical recommendations for selecting microwell plates, foundational principles of fluorescence microscopy, and fundamental approaches for developing and validating cell-based assays.

However, in this new white paper, we shift our focus from the theoretical to practical aspects of developing cell-based imaging assays. Development of cell-based assays demands meticulous attention to detail and a commitment to optimization. Here, we offer valuable insights, tips, and tricks to enhance the quality of your cell-based assays within the laboratory setting. By incorporating these optimization techniques, you can significantly elevate the impact of your work and drive advancements in the field of drug discovery and development.

Exploring Optimal Cell-Seeding Densities

Developing cell-based assays requires careful consideration of various parameters that can influence the outcome. Among these parameters, cell density plays a significant role and demands empirical testing to ensure accurate results. To facilitate comprehensive evaluation of assay parameters, microwell plates provide a convenient platform for arraying different test conditions. We recommend experimenting with at least three seeding densities, including the hypothesized optimal density, a substantially lower density, and a considerably higher density. This approach allows for a thorough exploration of the impact of cell density on assay outcomes.



Here are some additional tips to make the evaluation of optimal cell-seeding densities more effective and insightful:

1. Account for cell adhesion: Different cell types require specific surface conditions for optimal adhesion and growth. For a detailed discussion on selecting microwell plates with appropriate surface conditions, refer to our first white paper in this series on cell culture.
2. Conduct pilot experiments: Preliminary experiments using a range of cell-seeding densities can help determine the optimal range where cells exhibit ideal growth, maintain viability, and avoid issues like overcrowding or underrepresentation.
3. Assess cell viability: Utilize assays such as trypan blue exclusion or ATP-based viability assays to determine cell viability at different seeding densities. Avoid cell densities that lead to excessive cell death or stress.
4. Measure cell proliferation: Incorporate proliferation assays, such as BrdU or EdU incorporation, to evaluate cell growth at different densities. Select a density that allows cells to proliferate within the desired time frame of the assay.

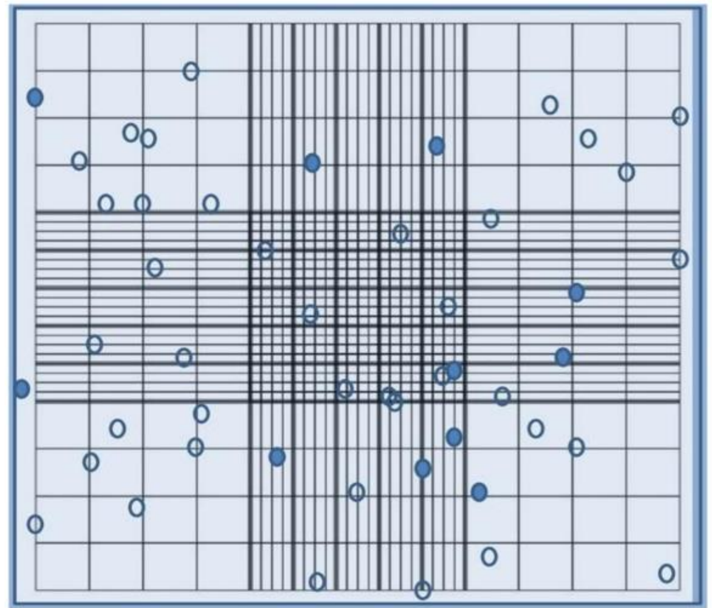
Mastering the Art of Cell-Seeding Densities: Achieving Consistency and Accuracy

Accurate and consistent cell-seeding densities are essential for reproducibility in cell-based assays. However, achieving consistent seeding densities across different runs of the same assay can be challenging. Estimating cell density in a suspension involves sampling from a population, introducing inherent statistical variability.

Automated cell counters offer efficient and precise cell counting compared to manual methods. However, their accuracy can vary based on factors like the specific automated counter used, cell conditions, and operator expertise.

Manual counting methods, involving a hemocytometer and a microscope, rely on visual identification and manual counting. Although time-consuming, this process can be improved by having two skilled operators independently count cells from two samples of the cell suspension.

On the other hand, automated cell counters utilize technologies like flow cytometry or image analysis algorithms to detect and count cells automatically. These systems provide faster results, reduce human error, and analyze large cell populations in a short timeframe, enhancing efficiency and minimizing variability between operators.



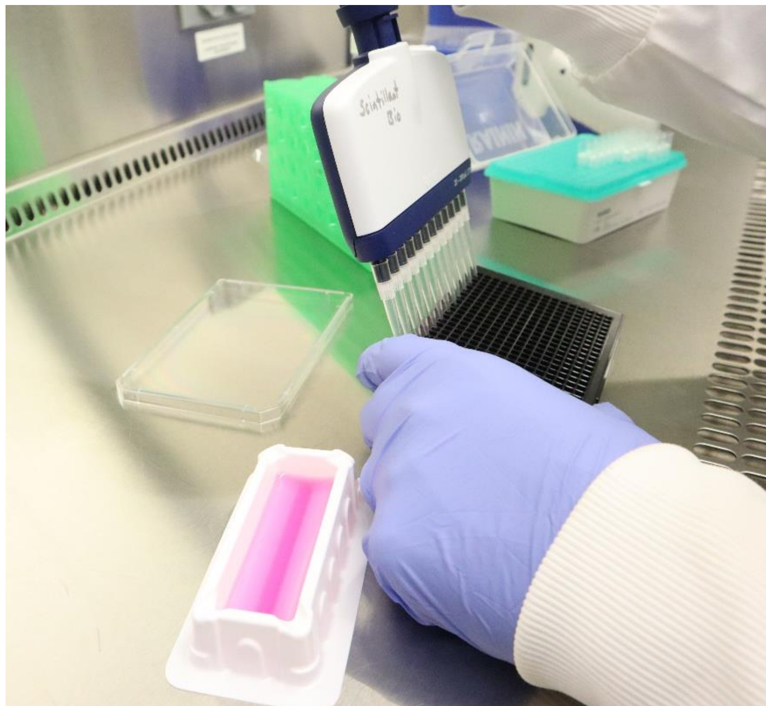
While automated cell counters are generally accurate for routine cell counting, several factors should be considered:

1. Dissociate cell clumps: Properly dissociating cell clumps ensures a homogeneous cell suspension, preventing uneven cell distribution and inaccurate seeding densities. Gentle pipetting after enzymatic dissociation can help break up clumps and passing a cell suspension through a cell strainer can eliminate cell clumps from the cell suspension used for seeding.
2. Statistical variability: Even with automated cell counters, statistical variability remains when sampling from the original cell suspension. Taking multiple samples and averaging the counts can mitigate this issue.
3. Calibration and validation: Following the manufacturer's instructions for calibration and validation is crucial to ensure accurate and reliable results from automated cell counters.
4. Cell types and conditions: Different cell types and conditions can pose challenges for automated cell counters. Irregular cell shapes, small cells or clumping may affect detection and counting accuracy.
5. Operator expertise: While automated counters reduce human error, operators still need to understand the instrument's operation and cell counting principles for correct usage and interpretation of results.
6. Verification: Periodically verifying results obtained from automated cell counters by comparing them with manual counting or alternative methods is advisable to confirm accuracy.

In summary, automated cell counters are generally accurate and efficient for cell-counting tasks. However, adherence to manufacturer guidelines, awareness of instrument limitations, and result verification are crucial for ensuring accuracy in specific experimental contexts.

Achieving Even Cell Distribution in Microwell Plates: Best Practices

Immediately after plating cells in microwell plates, particularly in 96-well plates, it is crucial to distribute the cells evenly across the wells. Placing the plates directly in the tissue-culture incubator or leaving them in a biosafety cabinet can lead to cell aggregation, especially at the well edges, due to vibrations.



To achieve optimal distribution in 96-well or 384-well plates, we recommend the following procedure: hold the plate with two hands and move it forward and backward several times, followed by side-to-side movements, repeating the entire process a few times. Avoid circular motions as they tend to cause cells to gather at the well edges. After distributing the cells, place the plate on a lab bench or an anti-vibration table for 20 - 30 minutes before transferring it to the tissue-culture incubator. These steps allow the cells to distribute relatively evenly across the wells, promoting consistent results.

Optimizing Timing & Duration of Compound Incubation and Assay Endpoints

The timing of the assay after cell seeding is another critical factor to optimize. Cells typically require some recovery time after seeding, with 16 - 24 hours being a common waiting period for adherent cells before running an assay. For imaging assays, it may take up to 48 hours for cells to assume their normal morphology in culture, which may be desirable for obtaining optimal cell images. To determine the ideal timing, we recommend preparing identical 96-well or 384-well plates and testing each at different time points.

Optimizing compound incubation time in cell-based assays is crucial for accurate and comprehensive evaluation of compound efficacy and toxicity. Whenever possible, we highly recommend employing extended incubation periods for compounds. This approach allows for testing compounds long after they have achieved binding equilibrium with their respective targets, ensuring that their biological effects are fully realized.

The rate at which a compound reaches equilibrium binding with its receptor depends on the association rate constant (k_{on}), the dissociation rate constant (k_{off}), and compound concentration. Compounds at lower concentrations require more time to reach equilibrium binding compared to higher concentrations. This consideration becomes particularly relevant when conducting dose-response testing.

Additionally, compounds targeting intracellular targets necessitate a longer incubation period to reach equilibrium binding compared to those binding to extracellular targets. The intracellular environment poses additional challenges that require sufficient time for compounds to traverse cellular barriers and effectively interact with their intended targets.

To develop robust cell-based assays, incorporating variable durations of compound incubation is advisable. This approach allows for the evaluation of changes in potency over time and potential alterations in toxicity profiles. By examining the effects of different incubation durations, researchers can gain valuable insights into the

temporal aspects of compound activity and toxicity, leading to a more comprehensive understanding of their pharmacological properties.

By optimizing compound incubation time, researchers can enhance the accuracy and reliability of cell-based assays, facilitating a more robust assessment of compound efficacy and safety profiles.

Summary and Conclusions

In order to obtain accurate and dependable results, it is crucial to explore the optimal cell-seeding densities, among other best practices for developing cell-based assays. By carefully considering cell density and conducting empirical testing with different seeding densities, researchers can comprehensively evaluate the impact on assay outcomes. In order to determine the optimal range for cell growth and assay performance, researchers should take into account several factors, including differences in cell adhesion, viability and proliferation across cell lines, cell types and plate types, taking into consideration plate surface and plate coating conditions. Empirical testing with pilot experiments is necessary to optimize cell-seeding density.

To ensure consistency and accuracy in cell-seeding densities, automated cell counters offer efficient and precise cell counting compared to manual methods. However, it is important to consider factors such as cell clumping, statistical variability, calibration and validation, cell types and conditions, operator expertise, and result verification to optimize the use of automated cell counters in specific experimental contexts.

Achieving uniform cell distribution in microwell plates is vital for obtaining consistent results. By following best practices, such as proper plate handling techniques and allowing cells to distribute evenly before incubation, researchers can minimize cell aggregation and ensure reliable data.

Optimizing the timing of compound additions and assay endpoints is essential for maximizing accuracy and gaining valuable insights into compound efficacy and toxicity. Extended incubation periods should be employed to allow compounds to reach binding equilibrium with their targets, ensuring the full manifestation of their biological effects. Understanding factors that influence binding equilibrium, such as compound concentration and target location, empowers researchers to design robust cell-based assays that provide a comprehensive understanding of compound pharmacological properties.

Considering the urgent need for more precise and reproducible biological research leading to groundbreaking therapeutics, the development and validation of accurate and reliable cell-based assays should be given high priority. By optimizing the parameters described in this paper, you can significantly improve your own research using cell-based assays.

We believe that by sharing this knowledge, we can foster a vibrant community of assay-development scientists who strive for excellence. We enthusiastically embrace this opportunity to enhance our collective expertise, push the boundaries of scientific discovery, and pave the way for innovative breakthroughs. Stay tuned for additional white papers in this series, and please don't hesitate to reach out to us with any questions or comments.

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