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Evaluation of Compound Storage Efficacy in DISPENDIX I.DOT HT and S Source Plates at -20°C and Room Temperature

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The Need for Correct Compound Storage to Maintain Stability

The maintenance of compound stability is essential to obtaining accurate, valid, and reproducible results. Incorrect drug storage can result in compound precipitation, degradation, or evaporation, all of which can influence dose-response curves and ultimately affect the validity of the results[1]. Factors influencing compound stability include the storage temperature – as this can affect rates of evaporation – and the type of plate – as compounds may interact or bind to certain materials[2]. Furthermore, successful compound dosing requires an accurate dispensing solution to ensure that the expected volume of the compound is added to the cells[3]. Effective storage and dispensing solutions are therefore key to ensuring precise dosing and accurate, reproducible results.

The I.DOT Non-Contact Dispenser for Accurate Dispensing

The I.DOT Non-Contact Dispenser is a liquid handler designed for reproducibility. It can accurately transfer volumes as low as 2.55 nL at a 0.1 nL resolution and has integrated volume verification where each droplet is detected and counted by an optical sensor before addition to the target plate. This ensures that the correct volume is being added every time. The I.DOT Liquid Handler comes in various forms, including the I.DOT S, which uses 96-well source plates, and the I.DOT HT, which uses 384-well source plates. The high-throughput nature of the I.DOT Non-Contact Dispenser makes it particularly effective for compound screening experiments, reducing human error, ensuring precise dispensing, and enhancing efficiency.

Storage Solutions to Maintain Compound Stability

Compound storage solutions allow for the storage of compound-loaded plates, such as lidded I.DOT source plates, in an environment optimized to maintain compound stability. The Roylan Developments StoragePod system is an innovative product designed to prevent moisture and oxygen from degrading sensitive compounds. It achieves this by using a flow of nitrogen to create a dry, low oxygen and moisture content storage environment, which is maintained until the StoragePod is accessed to retrieve compounds.
In this study, the suitability of I.DOT S and I.DOT HT source plates for maintaining compound stability was assessed. These source plates were tested in different conditions, including different storage solutions (the StoragePod system vs. a dry air environment and at different temperatures. The performance of the source plates was assessed by the treatment of cells with the stored compounds and subsequent tests of cell viability and growth.

Materials and Methods

To assess the ability of DISPENDIX I.DOT HT and I.DOT S source plates for compound storage, both types of source plates were prepared with compounds at defined concentrations. Cell proliferation assays were carried out using one of each of the HT.60 and S.100 plates at t=0 to establish baseline results. Following the completion of the experiment at day zero, sets of four plates were stored in different conditions. Two sets were placed in StoragePods, one set at -20°C and the second at room temperature (RT). A further two sets were placed in a dry air environment, one set at -20°C and the second at RT. After the storage period of two months (t=2), cell viability assays were performed on all the plates. The cell viability assays used were CellTiter Glo (CTG) assays from Promega. These are luminescent assays measuring cellular ATP levels. An incucyte (a live-cell imaging system) was used to assess cell growth, and growth curves were produced.

Results

Dose-Response Curve Evaluation
On experiment completion, the assay results from before (t=0) and after (t=2) the two-month storage period were compared. When stored at -20°C, the I.DOT HT showed a strong correlation between the t=0 and t=2 viability assays for both the StoragePod and the dry air environment (R = 0.97 and R = 0.95, respectively; Fig. 1). These data show that the StoragePod and dry air environment both maintain compound integrity for two months at -20°C when an I.DOT HT source plate is used, as cell viability is maintained.

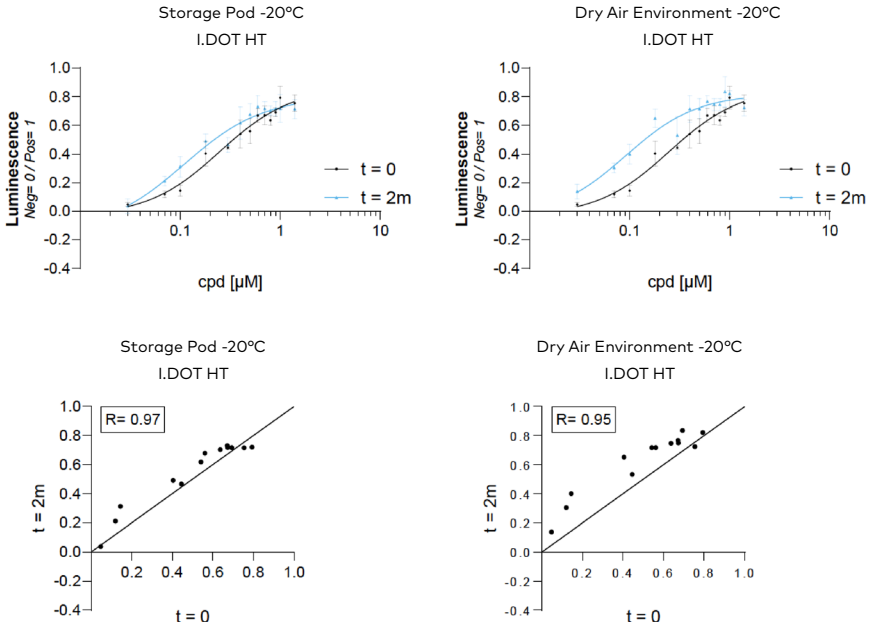

Figure 1. *Dose-response curves (top) and timepoint correlation determination (bottom) showing the effectiveness of the I.DOT HT in maintaining compound stability after 2 months at -20°C. (N=4)*

Plates stored at RT performed worse than at -20°C. Despite a good correlation between t=0 and t=2 (R = 0.92), plates stored in the StoragePod showed moderate compound degradation, as shown by decreased cell viability and an inability to fit a curve (Table 1 and Fig. 2). Plates stored in the dry air environment exhibited significant compound degradation with high dose-response variability resulting in a poor correlation between t=0 and t=2 (R = -0.77). These data suggest that compound storage is not effective at RT.

Table 1. *Parameters from the dose-response curves used to assess storage conditions showing compound degradation when stored at RT.*

Compound 2	I.DOT HT					I.DOT S				
	t = 0	StoragePod t = 2 mo		Dry Air Environment t = 2 mo		t = 0	StoragePod t = 2 mo		Dry Air Environment t = 2 mo	
		-20°C	RT	-20°C	RT		-20°C	RT	-20°C	RT
Top	0.76	0.80	n/a	0.82	n/a	0.73	0.75	0.66	0.75	n/a
EC50	0.22	0.11	n/a	0.09	n/a	0.11	0.10	n/a	0.02	n/a
R^2	0.91	0.91	n/a	0.86	n/a	0.90	0.90	0.56	0.83	n/a
R (0 vs. 2 mo)		0.97	0.92	0.95	-0.77		0.92	0.75	0.95	0.16
Curve fit										
Dispensing										

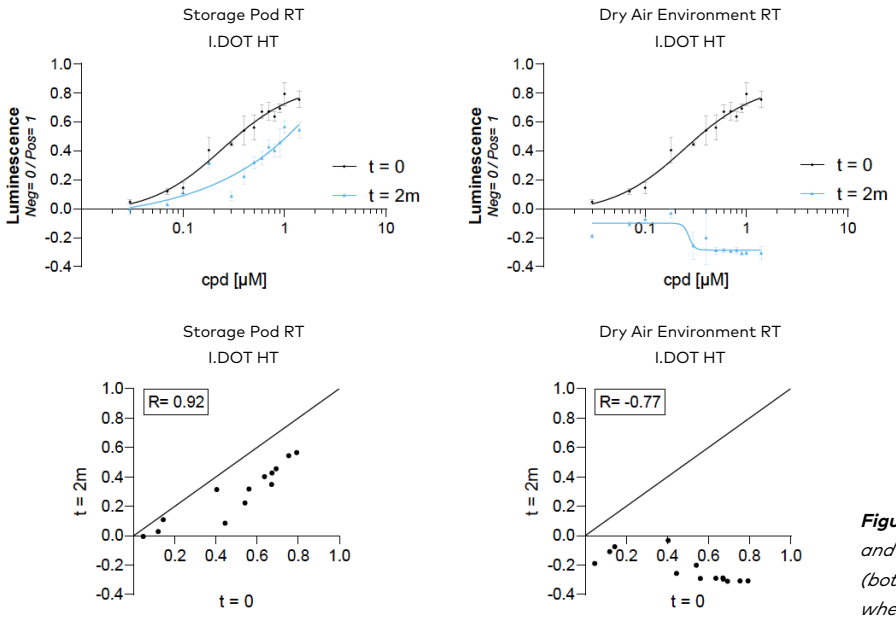

Figure 2. *Dose-response curves (top) and timepoint correlation determination (bottom) showing compound degradation when stored at RT. (N=4)*

Similar results were seen with the I.DOT S.100 plate. Storage at -20°C in the StoragePod or dry air environment produced similar dose-response curves to the baseline, with a high correlation between t=0 and t=2 (StoragePod: R = 0.92; dry air environment R = 0.95). Storage at RT performed worse than at -20°C with a poor correlation between t=0 and t=2 (StoragePod: R = 0.75; dry air environment: R = 0.16).

Dispensing Evaluation

Dispensing consistency was assessed using the I.DOT Liquid Handler's integrated droplet detection technology, which counts the number of droplets dispensed. Evaluation of the log files indicated whether drops were counted or missed.

Compounds stored in the dry air environment at RT showed repeated misses when stored in the I.DOT HT plates (**Fig. 3**). When stored at RT in the StoragePod, randomly distributed misses occurred. Compounds stored at -20°C performed well in both the Storage Pod and the Dry air environment with only a few randomly distributed misses, except for compound 1 in the Dry air environment, which seemed to experience issues.

Dispensing evaluation was also carried out after storage in I.DOT S plates. When stored in the dry air environment at RT, all of the compounds precipitated and caused a blockage, resulting in a complete failure to dispense. Compounds stored in the dry air environment at -20°C were successfully dispensed. Storage in the StoragePod resulted in successful dispensing at both RT and -20°C, except for some blockage at high concentrations where precipitation may have occurred.

Dispensing from the I.DOT HT and I.DOT S plates was, therefore, effective when compounds were stored at -20°C either in the dry air environment or the StoragePods, emphasizing the effectiveness of compound storage in the I.DOT source plates.

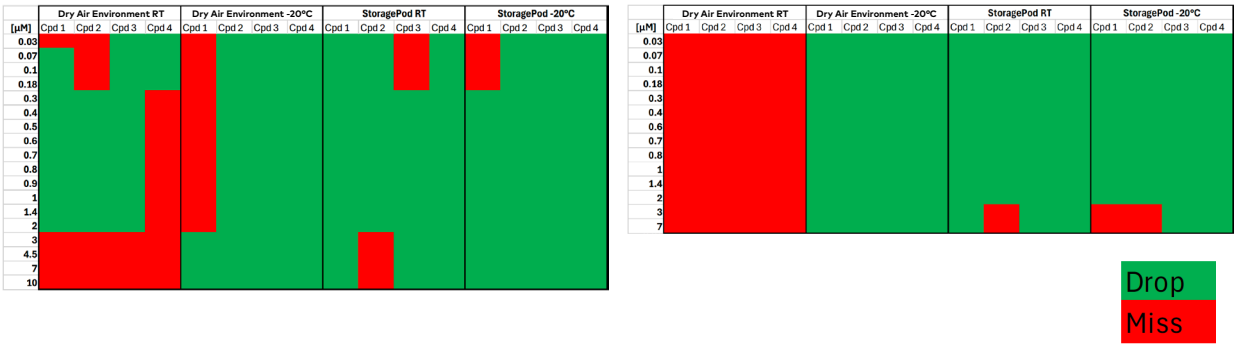

Figure 3. *Dispensing evaluation of (**a**) the I.DOT HT and (**b**) the I.DOT S showing mostly effective dispensing of compounds stored at -20°C, except for some randomly distributed misses and multiple misses when compounds are stored at RT.*

Growth Curve Evaluation

Growth curves, produced from images obtained from an incucyte, were used to further evaluate the effectiveness of compound storage in I.DOT source plates. At each compound concentration, compounds stored at -20°C in either the dry air environment or StoragePod produced growth curves comparable to the fresh compound (t=0; Fig. 4). In addition, end-point analysis showed no significant differences in cell viability after treatment with the compound stored at -20°C. Treatment with the compound stored at RT in the dry air environment significantly lowered cell viability ($p<0.0001$) at all concentrations. At lower concentrations, treatment with the compound stored at RT in the StoragePod also significantly lowered cell viability ($p<0.05$). These data further support the effectiveness of compound storage at -20°C in I.DOT source plates.

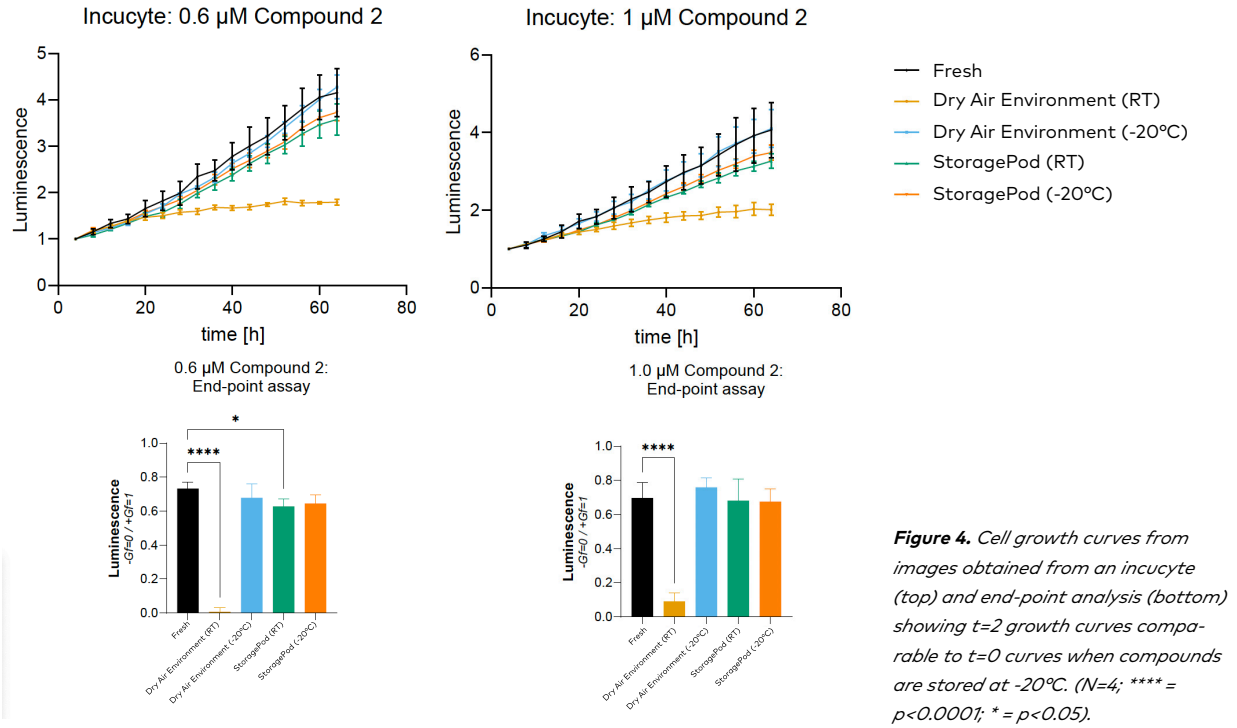

Figure 4. *Cell growth curves from images obtained from an incucyte (top) and end-point analysis (bottom) showing t=2 growth curves comparable to t=0 curves when compounds are stored at -20°C. (N=4; **** = p<0.0001; * = p<0.05).*

Discussion

The success of the cell viability and growth curve experiments when carried out using compounds stored at -20°C for two months highlights the robustness of the I.DOT HT and S plates for compound storage over prolonged periods at -20°C. Although both the StoragePod and dry air environment provide environments suitable for storage at -20°C, the StoragePod outperforms the dry air environment at RT, both by minimizing compound degradation and maintaining dispensing precision. Minimal differences were seen between the I.DOT HT and I.DOT S source plates, indicating that both are equally effective for compound storage. Under appropriate conditions, the I.DOT system successfully maintains compound stability and ensures reproducibility in dose-response experiments.

Ensuring compound stability through using effective storage conditions is key to the validity, accuracy, and reproducibility of experiments, including dose-response, cell-based assays, and drug discovery. The system validated here could be used in experiments such as these, providing confidence that compounds are being stored successfully and will produce comparable results, even after two months of storage.

Conclusion

To obtain accurate, reproducible results, it is essential to consider storage conditions when carrying out the long-term storage of potentially unstable compounds. Both the I.DOT HT and S source plates are suitable for effective compound storage at -20°C in combination with either the StoragePod or dry air environment storage solutions. If RT storage is necessary, the controlled atmosphere provided by the StoragePod is advantageous over dry air environment systems. This study emphasized the importance of choosing the correct storage system to maintain compound stability and ensure reliable results.

The Cultivated B

The Cultivated B (TCB) is driving the transformation of food and food ingredient manufacturing towards a sustainable future. By developing specialized bioreactors and fermenters while applying innovative technologies such as precision fermentation and molecular farming, TCB accelerates the commercialization of alternative proteins economically and at scale. TCB's comprehensive portfolio of products and services is tailored to the needs of innovative food, supplements, and advanced materials companies. TCB works with companies to embed these sustainable technologies on local, regional and global scales, with operations in Europe and North America and a robust network of strategic partners in the Middle East, East Asia and Latin America. For more information, visit www.thecultivatedb.com and follow the company on LinkedIn.

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