



# Examination of Storage Conditions for Preservation of Pre-Plated DMSO Nanospots in ChemLib 1536-well and 3456-well Plates

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## ABSTRACT

For ultra-high throughput screening, 10 to 30 nL of compound dissolved in DMSO is spotted into 1536- and 3456-well ChemLib plates (Aurora Discovery, Inc.) and stored appropriately for a short time before screening. Although this practice eliminates the compound plating bottleneck, assay plates initially containing a known volume of DMSO slowly evaporate. Since many assays are sensitive to DMSO concentrations, even slight evaporation may cause intra-plate variation and thus decrease assay quality. Using a CYP3A4 Vivid Blue assay (Invitrogen), we investigated the rate, pattern, and quantity of evaporation over a one year time frame to identify best practices for long-term (i.e., six months or greater) storage of compound plates. Our method of study and findings for both 1536-well and 3456-well ChemLib plates follow.

## MATERIALS & METHODS



Piezo Sample Distribution Robot (PSDR), Aurora Discovery Inc., San Diego, CA

### Test plate preparation:

- PSDR used for assay plate preparation
  - 20 nL of 75:25 DMSO:H<sub>2</sub>O in 1536-well plates
  - 10 nL of 75:25 DMSO:H<sub>2</sub>O in 3456-well plates
- removed static and dust from plates with air 'knife'
- filled evaporation wells (when used) with 5  $\mu$ L (1536-well) or 1.5  $\mu$ L (3456-well) of 75:25 DMSO:H<sub>2</sub>O
- for bagged conditions, plates were individually bagged along with a dish containing 3 mL 75:25 DMSO:H<sub>2</sub>O

### Storage conditions:

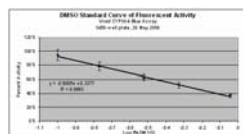
- 1: -20°C, without bag
- 2: -20°C, without bag, with evaporation wells
- 3: -20°C, with bag
- 4: -20°C, with bag, with evaporation wells
- 5: -20°C, with bag, with heat seal
- 6: -80°C, with bag
- 7: -80°C, with bag, with evaporation wells

### Curve plate preparation:

- PSDR also; substituted PicoRAPTR (Picoliter RAPid Transfer Robot) when not available
  - 0, 4, 16, 24, and 36 nL of 75:25 DMSO:H<sub>2</sub>O for 1536-well study
  - 0, 2, 3, 5, 9, and 18 nL of 75:25 DMSO:H<sub>2</sub>O for 3456-well study
- stored plate as follows:
  - RT in a bag for same-day usage
  - -20°C in a bag with a dish for next day usage

### Assay execution:

- FRD used to dispense assay reagents
  - 3.0  $\mu$ L reaction in 1536-well format
  - 1.5  $\mu$ L reaction in 3456-well format
- reactions consisted of:
  - 5 nM CYP3A4 enzyme
  - 20  $\mu$ M CYP3A4 Blue (BOMCC) substrate
  - 100  $\mu$ M NADP+
  - 1X regeneration system
  - 1X reaction buffer
- reactions were incubated 20 min. at RT prior to stop
- stop mix consisted of 30  $\mu$ M ketoconazole
- tcPR used for reading plates immediately after stop
  - Ex = 400 nm; Em = 460 nm
- data was analyzed using MS Excel
  - constructed standard curve of percent activity vs. log<sub>10</sub> of DMSO concentration
  - calculated percent activity for conditions by extrapolation from a DMSO-sensitivity curve:



### Method Notes:

- Reagents were prepared essentially as described in the Invitrogen literature
- A chiller adapted with a custom water jacket was used to maintain the enzyme mix at 4°C
- Plates were assayed in the same order at each time point
- CYP3A4 enzyme, substrate, NADP+, regeneration system, reaction buffer, distilled water, and PBS were obtained from Invitrogen; DMSO and ketoconazole from Sigma-Aldrich; anhydrous acetonitrile from Mallinckrodt.



Flying Reagent Dispenser (FRD), Aurora Discovery Inc., San Diego, CA



Topology Compensating Plate Reader (tcPR), Aurora Discovery Inc., San Diego, CA

## PRELIMINARY RESULTS—1536-well Assay

### Storage Over Time, All Conditions

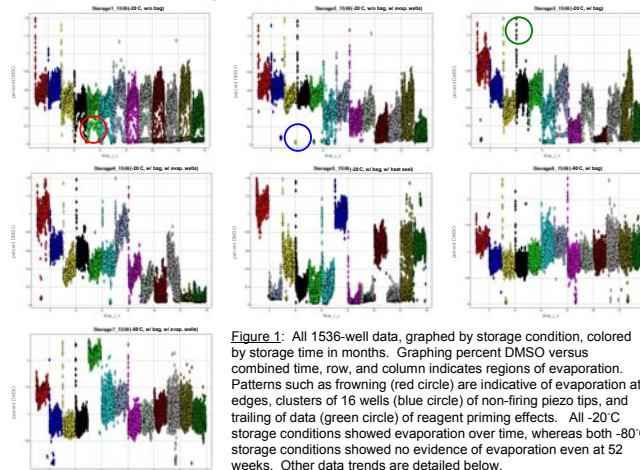


Figure 1: All 1536-well data, graphed by storage condition, colored by storage time in months. Graphing percent DMSO versus combined time, row, and column indicates regions of evaporation. Patterns such as frowning (red circle) are indicative of evaporation at edges, clusters of 16 wells (blue circle) of non-firing piezo tips, and trailing of data (green circle) of reagent priming effects. All -20°C storage conditions showed evaporation over time, whereas both -80°C storage conditions showed no evidence of evaporation even at 52 weeks. Other data trends are detailed below.

### Other Helpful Graphs

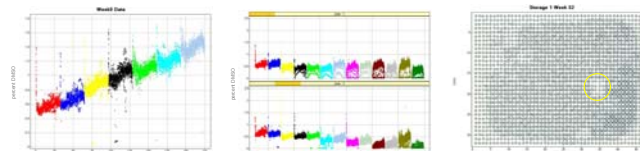


Figure 2: Left—apparent gain in DMSO across plates within a single run due to loss of enzyme activity over time. Such data trends, which confound DMSO amounts with enzyme activity, varied from month-to-month (see 3456-well data), thus smoothing data to quantify evaporation was not possible. However, predictable patterns present in the data do allow for qualitative determinations. Middle—data from storage conditions 1 and 2, colored by storage time in months. As in Figure 1, the graph shows percent DMSO versus combined time, row, and column to indicate regions of evaporation, but is trellised by assay order to help differentiate between evaporation and enzyme activity effects. This pair-wise comparison indicates that both conditions show evaporation—condition 1 at the edges and condition 2 across the whole plate. Right—data from storage condition 1 after one year, with wells sized by DMSO amount. An outer ring of evaporated wells is apparent, along with a cluster of 16 wells (yellow circle) corresponding to a non-firing piezo tip.

## CONCLUSIONS

For integrity of compound solution, some DMSO must be present in the well. In practice, effects upon data due to evaporation can be 'smoothed' out when using DMSO-sensitive reagents. We define a plate's 'drop-dead date', therefore, as the time at which no DMSO remains in some significant subset of plate wells, but ideally, at least half of the pre-spotted DMSO would remain when the plate is used in an assay.

Stored at -20°C, without a bag, ChemLib 1536-well plates (containing 20 nL 75:25 DMSO:H<sub>2</sub>O) would be usable for two months. With the addition of evaporation wells, half of the original DMSO appears present at seven months. Interestingly, storing plates with a bag but without filled evaporation wells is worse than filling the evaporation wells but not using the bag—plates stored at -20°C with a bag are useable for four months, and for seven months with filled evaporation wells. The -20°C heat-sealed condition was highly variable, with six good plates and six bad plates in no particular order. Re-centrifuging plates prior to assays might have improved the results, but was not done because of practical considerations. Finally, both -80°C conditions exhibited no discernable evaporation over the entirety of the 12 month study, likely due to the 75:25 DMSO:H<sub>2</sub>O freezing. Accordingly, such a practice would be ideal for shipping, provided one freeze-thaw cycle is permissible.

## PRELIMINARY RESULTS—3456-well Assay

### Storage Over Time, All Conditions

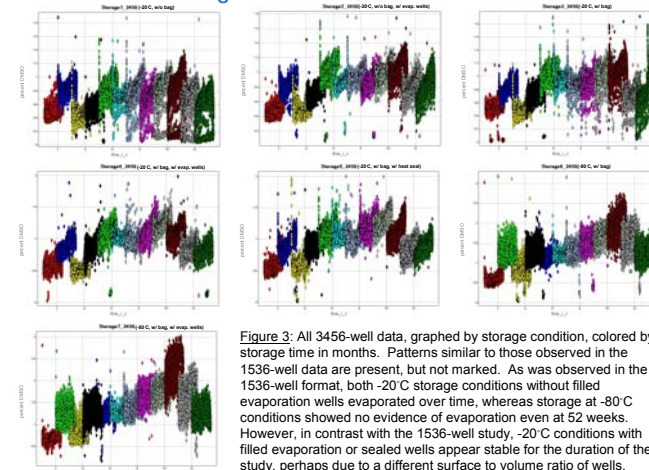


Figure 3: All 3456-well data, graphed by storage condition, colored by storage time in months. Patterns similar to those observed in the 1536-well data are present, but not marked. As was observed in the 1536-well format, both -20°C storage conditions without filled evaporation wells evaporated over time, whereas storage at -80°C conditions showed no evidence of evaporation even at 52 weeks. However, in contrast with the 1536-well study, -20°C conditions with filled evaporation or sealed wells appear stable for the duration of the study, perhaps due to a different surface to volume ratio of wells.

### Other Helpful Graphs

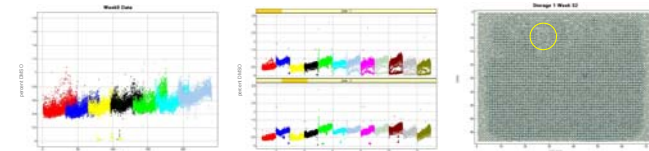


Figure 4: Left—apparent gain in DMSO within a single run due to loss of enzyme activity over time. Note that the effect is markedly less than that shown in Figure 3 (percent DMSO scales are identical), thus highlighting the observed trend variation and consequent impossibility of data smoothing. Middle—data from storage conditions 1 and 2, colored by storage time in months. In contrast with the corresponding 1536-well data, 3456-well storage condition 1 is evaporating and 2 is not. Right—data from storage condition 1 after one year, with wells sized by DMSO amount. An outer ring of evaporated wells is apparent along with a cluster of 36 wells (yellow circle) likely corresponding to under-dispensing piezo tips. (Despite rigorous daily quality control practices, tip-to-tip variance does add a level of complexity to this data.) In contrast, data from storage 2 (not shown) does not have the characteristic outer ring pattern, thus increasing confidence in the assessment that evaporation has not occurred.

## CONCLUSIONS

Stored at -20°C, without a bag, ChemLib 3456-well plates (containing 10 nL 75:25 DMSO:H<sub>2</sub>O) would be usable for five months. With the addition of evaporation wells, it appears that a full year of storage is possible—even without a bag. Plates stored at -20°C with a bag are useable for up to six months or so, beyond which variability appears to increase markedly, but with filled evaporation wells, the plates appear more consistent and useable for all 12 months. The -20°C heat-sealed condition was much more consistent than in the 1536-well study, with plate stability apparent for a year as well. Finally, as with the 1536-well study, both -80°C conditions exhibited no discernable evaporation over the entirety of the 12 month study.

## REFERENCES

Invitrogen Corporation (2005), *Vivid CYP3A4 Screening Kit Protocol*. (Invitrogen Corporation, Carlsbad, CA).

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