Validation of Low Volume 1536 "Assay Ready" Compound Plates for a Nuclear Hormone Receptor Screen.

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Abstract

The predispensing of compound libraries by acoustic droplet ejection is a convenient way of preparing "assay ready" compound plates. This method also allows for the direct (i.e. no predilution necessary) and precise dispensing of the low volumes of compound DMSO solution required for assays in 1536 plates. However, questions arise about the effects of time, temperature and dispensed volume on assay performance and repeatability. These issues about the "shelf-life" of assay ready plates are of particular importance if large batches of plates need to be prepared several days in advance, e.g. for HTS screens.

In an effort to validate this method of compound plate preparation for a particular screen and compound set, we predispensed agonists of a nuclear hormone receptor in 25, 75 and 150 nl volumes of DMSO in triplicate 1536 plates at different time points prior to performing a TR-FRET agonist assay (plates prepared 2 weeks, 1 week, 2 days, 1 day, 0 days before and stored sealed at room temperature). In addition, we kept a 2 week old plate set at –18 ºC to see if storage temperature had an effect on compound activities. We present a comparison and interpretation of the assay potency and efficacy results between these plate sets.

Methods

Preparation of test sets of predispensed ("assay ready") compound plates:

For comparison of the effects of storage time, temperature and dispensed volume, we determined the potencies (pEC_{50}s) and efficacies of 157 compounds in a coactivator recruitment agonist assay for a nuclear hormone receptor. Only results for compounds with efficacies between 3xSTDEV of control (DMSO) and 5x 100% control are included in the analysis. The preparation of assay ready compound plates was done very similar to the standard process used to prepare low volume assay ready compound plates at AstraZeneca R&D Mölndal. The compounds were prediluted in seven dilution steps to give a compound dilution source plate. From this plate, triplicate sets of plates were each prepared for three dispensed volumes and at different time points prior to the screen (see table). All plates were sealed and stored at 20ºC except for three plate sets that were kept at -18 ºC. The 25nl plates were dispensed without any dilutions; the 75nl and 150nl assay plates contained predispensed DMSO (50nl and 125nl, respectively) to which 25nl compounds from the source plate were added. The assay ready compound plates were spun and heat-sealed immediately after the dispense.

Sealing of plates: Velocity11 PlateLoc with Easy peel heat seal
Compound dispensing: Labcyte Echo555
Centrifugation of plates: Velocity11 Vspin
Source plates: 1536 Costar Cyclo-olefin copolymer (COC) Shallow Flat clear 3730
Assay plates: 1536 Greiner PS Shallow Flat White 782075

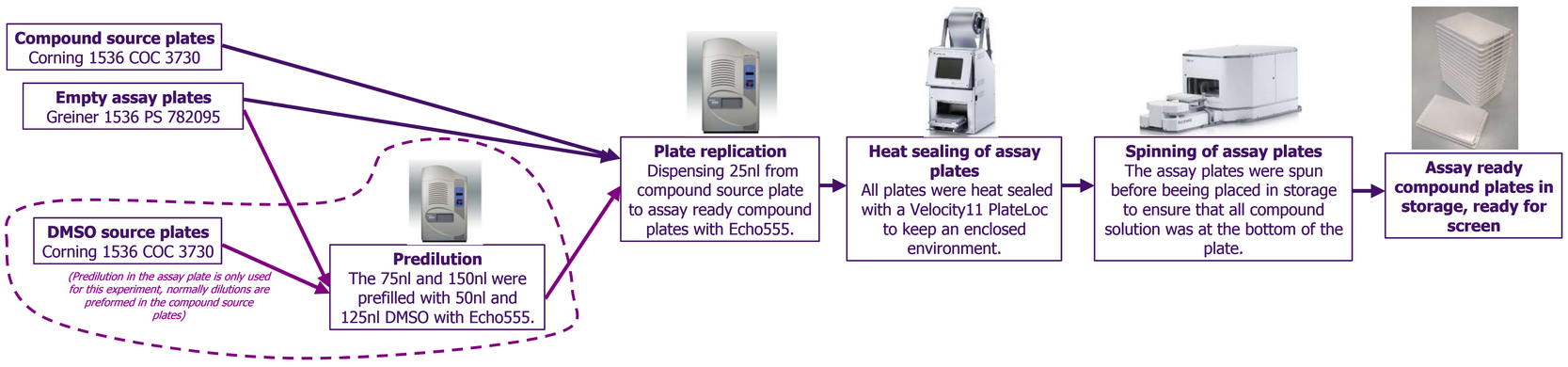


Fig. 1: Low volume assay ready compound plate workflow

Plate Set	Storage time	Storage temp.	Dispensed Vol.
1A, 1B, 1C	2 weeks	20 °C	25, 75, 150 nl
2A, 2B, 2C	2 weeks	-18 °C	25, 75, 150 nl
3A, 3B, 3C	1 week	20 °C	25, 75, 150 nl
4A, 4B, 4C	2 days	20 °C	25, 75, 150 nl
5A, 5B, 5C	1 day	20 °C	25, 75, 150 nl
6A, 6B, 6C	0 day	20 °C	25, 75, 150 nl

Table: plate sets prepared for comparison tests

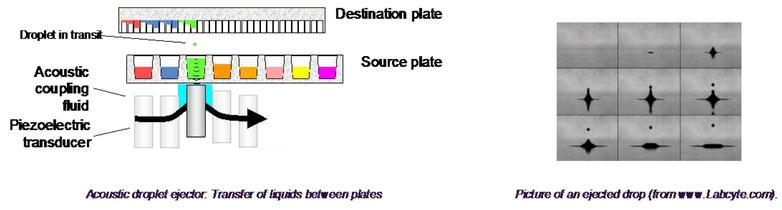
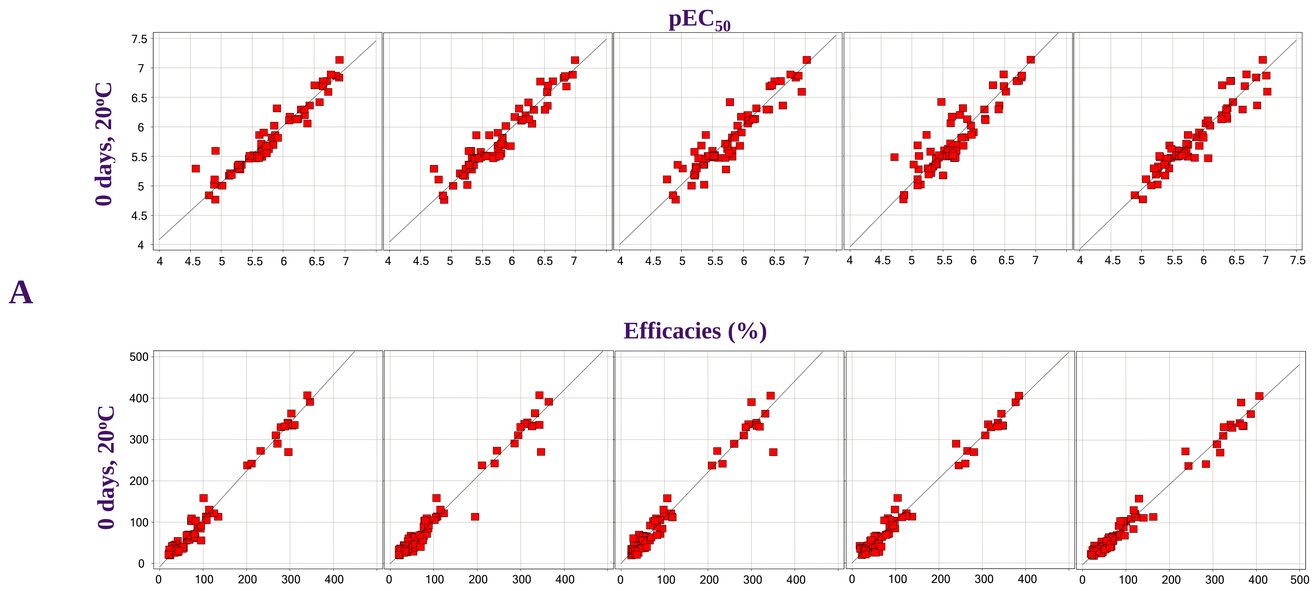


Fig. 2: Plate replication by acoustic droplet dispensing
The preparation of the assay ready compound plates was performed with the Echo555 acoustic dispenser which focuses ultrasonic acoustic energy into a fluid sample to eject small droplets of liquid from open wells. This makes it possible to transfer small volumes (nano litres) of liquids between plates.

Results

1. **Influence of storage of predispensed "assay ready" plates on compound potencies (pEC_{50}s) and efficacies.** Prolonged storage of compound solutions in DMSO in small volumes could potentially affect assay results, such as pEC_{50}s and efficacies (e.g. by adsorption of compounds to the plastic material of the plates, or by oxidation).
   In spite of the very low volumes of compound solutions in the wells, neither storage time nor temperature seemed to have major impacts on the screening results; however, a small decrease of reproducibility of pEC50s was seen at the 2 week timepoint which was less pronounced in the frozen (-18ºC) samples.



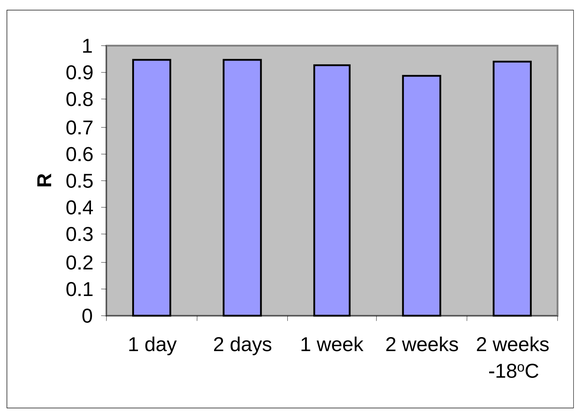


Fig 3: A, Scatter plots of potencies (pEC_{50}s) and efficacies (% of control) determined for the compound sets at the various storage times and temperatures indicated. The dispensed volume of compound solution was 25nl. For pEC_{50}s, the correlation coefficient R (orthogonal fit) is shown in B; R for the efficacy plots was between 0.98 and 0.99.

2. **Influence of dispensed volumes**. Low volumes of compound solutions may be more easily affected by evaporation of the solvent, which in turn could affect adsorption to the plate, re-dissolution of the compounds etc. As the data below show, there does not appear to be a great effect of dispensed volume for most compounds on pEC_{50} or efficacy.

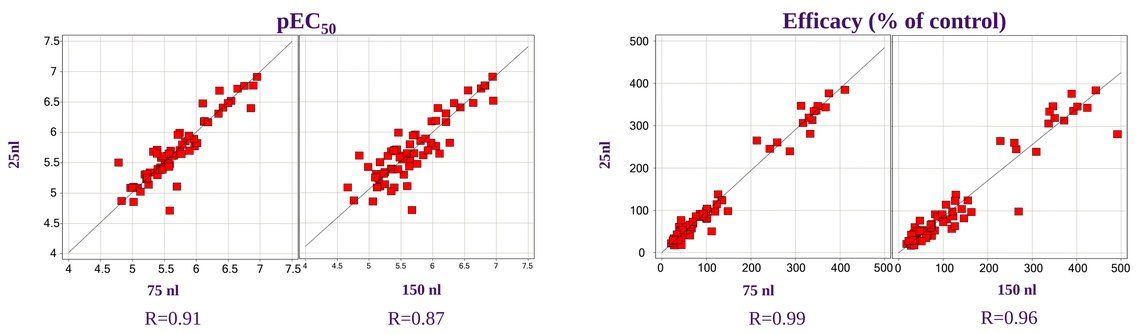


Fig. 4: pEC_{50}s and efficacies for dispensed volumes of 25, 75 and 150 nl; storage time 2 weeks; temp. = 20 ºC.

Summary:

1. Potencies and efficacies appeared to be fairly reproducible over a wide range of values in spite of variations of storage time, dispensed volume and temperature.
2. "Assay ready" compound plates can be prepared in advance of screening up to two weeks without significant effects on the screening results.
3. Further tests are needed to be able to expand the findings to other compound sets and assays.