Technical Note: 08004

The Use of Thermo Scientific PocketTips® to Reduce the Occurrence of Compound Precipitation

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The following procedure outlines a case study performed to compare the occurrence of compound precipitation using traditional pipetting methods with Thermo Scientific PocketTips by measuring absorbance.

Introduction

PocketTips, standard format automation tips compatible with many industry standard automated liquid handling platforms, are designed with an internally molded capillary pocket that holds volumes of 50 nl, 100 nl, 250 nl or 500 nl. The use of PocketTips for laboratory procedures, such as compound screening, eliminates intermediate aqueous dilution steps traditionally performed to reduce the DMSO concentration used to store compound samples. These intermediate dilution steps often cause compound instability and precipitation resulting in false positives and unreliable data. PocketTips, by eliminating these intermediate steps, keep compounds stable, thus generating more reliable, stable and reproducible assay data.

Materials

1. 10 µl DMSO Compound
2. P450, Low-DMSO Tolerant Enzymatic Assay
3. Thermo Scientific PocketTips for Biomek FX 384-Channel Head, 100 nl pocket (Item no. FX384P30-100)
4. Beckman® MultimekTM and Biomek® FX
5. 384-Well Microplate containing DMSO
6. 384-Well Microplate containing aqueous buffer
7. 384-Well Microplate

Methods

There are 14 compounds per plate and each compound is done in duplicate, comprising 2 rows and 10 columns of a standard SBS 384-well microplate (for example, A5-B14 is a single compound; 4 rows and 10 columns (M15-P24) are control wells; columns 1-4 are excluded).

Traditional Method to Determine Base Line Precipitation

1. DMSO Dilution Plate: 10 µl of DMSO compound is diluted in a ratio of 1:3 in DMSO (for example, top point is 10 µM, next point is 3.33 µM, etc.)
2. Aqueous Dilution/ Intermediate Plate: Transfer 5 µl from the dilution plate into aqueous buffer
3. Assay Plate: Transfer 5 µl from dilution plate into the assay plate on the Beckman Coulter Multimek (for example, top point is 50 µM, next point is 16.67 µM, etc.)
4. Read plate using absorbance algorithm to determine precipitated wells

Procedure Performed with PocketTips

1. DMSO Dilution Plate: 10 µl of DMSO compound is diluted in a ratio of 1:3 in DMSO (for example, top point is 10 µM, next point is 3.33 µM, etc.)
2. Assay Plate: Transfer 100 nl from the dilution plate into the assay plate using PocketTips and perform in-tip mixing on the Beckman Coulter Multimek or Biomek FX (for example, top point is 50 µM, next point is 16.67 µM, etc.)
3. Read the plate using an absorbance algorithm to determine what wells precipitated

Results

Traditional Method

1. 47 data points lost due to precipitation
2. 14 compounds lost at least one data point

3. 10 compounds lost two or more data points

PocketTips Procedure

1. 5 data points lost due to precipitation
2. 12 compounds exhibited no precipitation
3. Only 2 compounds lost two or more data points

With the traditional procedure, there were multiple compounds precipitating at various molarities (see Figure 1). A well showing excessive precipitation would prevent determining an accurate IC50 value (potency value) and data point is excluded from IC50 curve.

PocketTips greatly reduced, and in some cases, eliminated altogether the precipitation issue. Figure 2 shows the results of the PocketTips procedure, in which 85.8% of compounds did not precipitate.

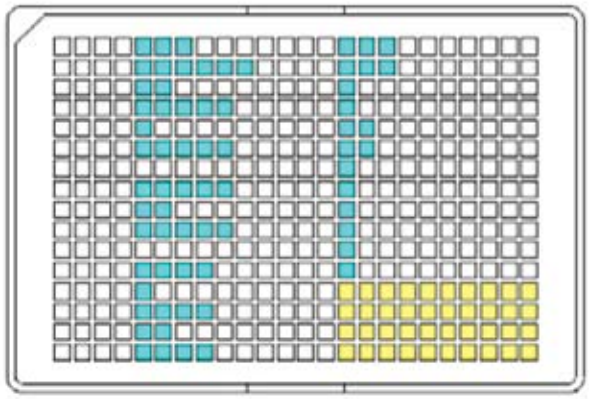

Figure 1:
Plate Representation of Traditional Method

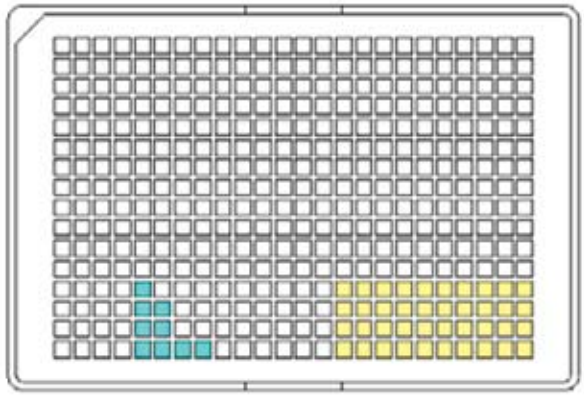

Figure 2:
Plate Representation of PocketTip Method

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