

Effect of Plate Mixing, Fluid Addition Height and Speed on Reducing Addition Artifacts and Negative Control Drift Using the FDSS6000

Introduction

Two artifacts often present following fluidic addition to wells are: 1) Sharp change in signal (either up or down) not associated with a biological response and 2) Drifting of the negative control well signal over the time course of the assay. Such artifacts are reduced using software algorithms including negative control correction (CeuticalSoft, Hudson, NY). However, the versatility of FDSS6000 hardware allows scientists to address addition artifacts, a more elegant solution than post assay data manipulation.

In this study we compare the effects of cell plate shaking, pipettor fluid addition height, and pipettor fluid addition speed on reducing artifacts and improving assay response, using the FDSS6000.

Materials and Methods

A No Wash Membrane Potential Kit was used in the study. Directions for use were followed per kit instructions. Briefly, cells were seeded overnight in 25 μL volume. The next day 25 μL no wash reagent was added to cells. Following a thirty minute incubation at RT 13 μL was added offline. The second 13 μL addition was made on the FDSS6000 and the data collected for 5 min in 1.5 sec intervals. Plate mixing speed was set at 6.

Results

Table 1: Description of the three groups. Addition heights 6 mm and 7.5 mm corresponds to 55 μL and 75 μL above the cell layer, respectively.

Group	Plate Mixing	Addition Height (mm)	Addition Speed ($\mu\text{L}/\text{sec}$)
1	NO	6	13
2	YES	6	7
3	YES	7.5	7

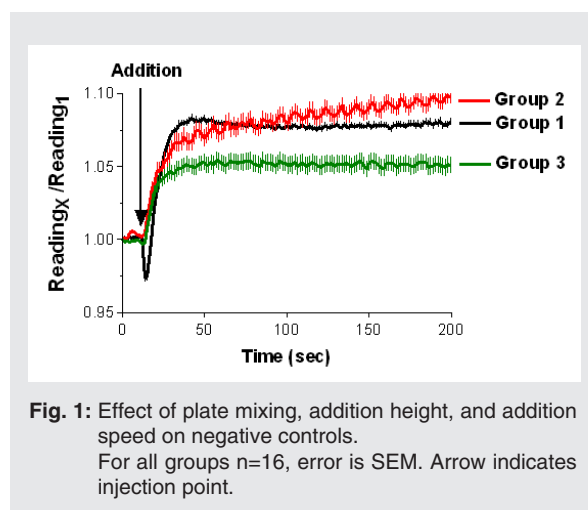


Fig. 1: Effect of plate mixing, addition height, and addition speed on negative controls. For all groups $n=16$, error is SEM. Arrow indicates injection point.

In Figure 1 note the addition artifact (drop) immediately following fluidic addition in Group 1. Plate mixing removes this artifact (Groups 2 and 3). Addition speed 13 $\mu\text{L}/\text{sec}$, mixing 'on' also eliminated the addition artifact (data not shown). Increasing the pipettor height to 7.5 mm (Group 3) decreased the plateau height of the negative control response along with reducing the rate of signal increase over time, as compared to Group 2.

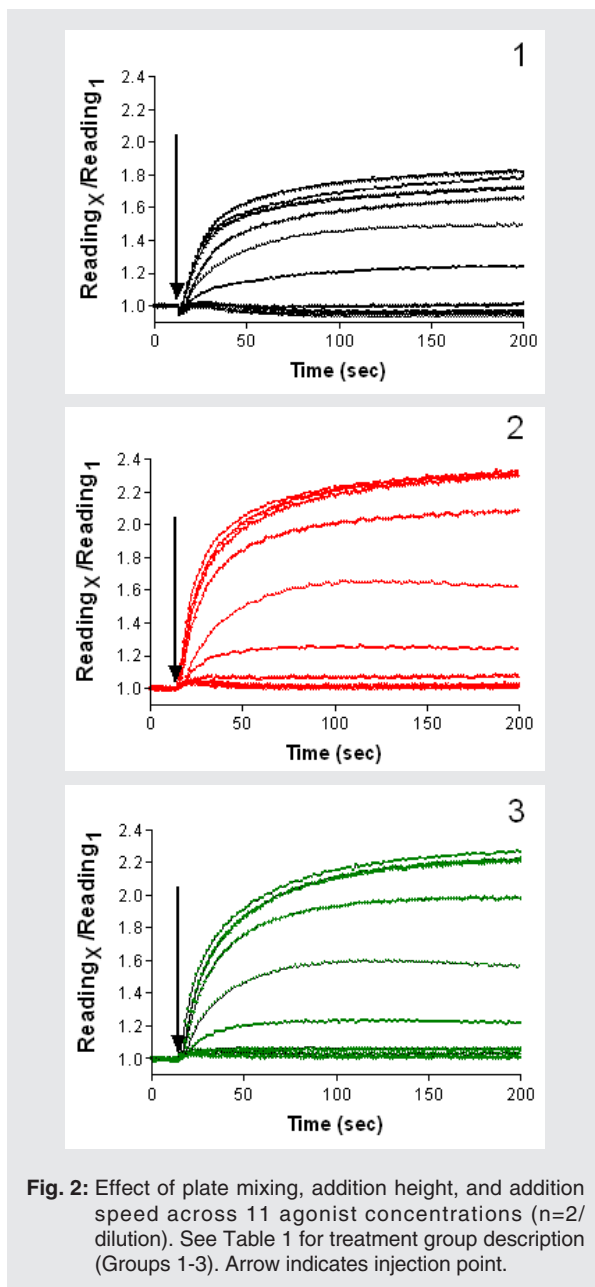


Figure 2 shows the effect of mixing, pipettor height, and injection speed on the agonist mediated response. Note the increase in the signal: background ratio, from 1.8 (Group 1) to 2.3 (Groups 2 and 3). Further, with Groups 2 and 3 there are three dilutions distinct from max and min response; by contrast Group 1 has only 2 distinct dilutions.

Summary

Plate mixing eliminated the signal drop associated with fluid addition along with increasing assay dynamic range. Increasing addition height decreased the maximal change in signal of the negative control and stabilized the signal over time, as compared to a lower addition height.

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