

Cell Plate Mixing versus Pipet Mixing A Comparative Study on Reducing DMSO Artifacts and Improving Assay Performance Using the FDSS6000

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Abstract:

DMSO-mediated artifacts remain an important source of error in cellular assays. Rapid homogenization of DMSO into water requires active mixing, else DMSO (and compound) may fall directly onto adherent cells (1). The resulting cellular response may be greater than expected owing to spuriously high DMSO/compound concentration(s). There are several methods to deal with DMSO artifacts: 1) Employ data processing algorithms to mathematically force the DMSO-only control well traces to a horizontal line. 2) Mechanically mix the compound/DMSO solution using the pipettor. 3) Mix the cell plate. In the present study we determined the effect of pipet mixing, cell plate mixing, and data processing on an assay using a no wash membrane potential dye, KCl, and a hyperpolarizing compound. The results suggest cell plate mixing (1) reduces DMSO-mediated artifacts and (2) reduces cellular response levels to predicted levels, based on known compound concentrations.

Materials/Methods:

An adherent cell line expressing a recombinant potassium channel was seeded at 250,000/mL, 50 μ L, into Poly-D-Lysine coated plates. Cells were incubated overnight at 37°C, 5% CO₂. The media was completely removed by "flicking" and 50 μ L no wash MP dye added to each well. The cells were incubated for 15 min incubation at 37°C and before analysis. Buffer was supplemented with 3% DMSO and used to dilute KCl to 150 mM, 50 mM, and 17 mM. The hyperpolarizing compound was similarly prepared to 30 μ M, 10 μ M, and 3 μ M. The FDSS6000 384 well pipetting head delivered 25 μ L reagent to cells at 10 sec (marked with an arrow). Plate mixing and pipet mixing were used as indicated in the results. The exposure time was set approximately 200 msec and readings were taken every 0.5 sec. Results are reported as the ratio of reading at time X (Rx)/reading 1 (Ro). Bias subtraction was set at 1 sec after reagent addition. Data was analyzed using CeulicalSoft, Hudson, NY or Prism 3.0 (GraphPad). For negative control correction the means for each reading of control wells was calculated. From this the ratio of reading 1/reading X was calculated and multiplied against the readings of all wells at the appropriate reading time. For Concentration Response Curves the reading at 40 sec (30 sec reaction time) was used.

Fluidic Mixing Affects the Kinetic Response to a Hyperpolarizing Compound

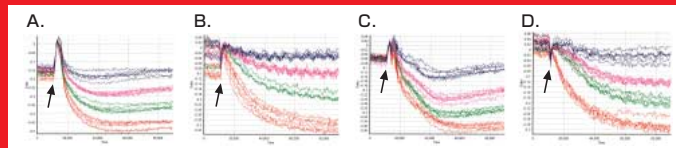


Figure 1. Three concentrations of hyperpolarizing reagent (Red =10 mM, Green = 3.3 mM, Purple =1.1 mM) plus DMSO-only (Blue) were assayed with the following hardware parameters: (A) Cell plate mixing OFF and pipet mixing OFF (B) Cell plate mixing ON and pipet mixing OFF (C) Cell plate mixing OFF and pipet mixing ON (D) Cell plate mixing ON and pipet mixing ON.

Note the DMSO artifact immediately after addition (A); the artifact could be a result of cellular depolarization or incomplete DMSO mixing. In any case with plate mixing on the DMSO artifact disappears (B). With pipet mixing the DMSO artifact, along with a pipet mixing artifact (a dip in signal at 15,000 ms) is present (C). Pipet mixing plus cell plate mixing (D) gives results similar to panel B.

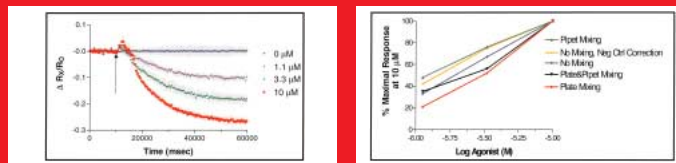


Figure 2. Data from Figure 1 (A) presented with negative control correction. Data presented as the mean±SD of six replicates.

Figure 3. The net changes (DMSO-only subtraction) of the three hyperpolarizing reagent concentrations were calculated from data in Figures 1 and 2. The maximal response (using 10 μ M) for each group was set at 100%; concentrations 3.3 and 1.1 μ M are reported as percent activity of 10 μ M. (Ideally the expected response should be 100%, 33%, and 11 %). With no mixing (blue) the response decreases to about 40 % maximal response. With plate mixing (red) the response decreases to about 20 % maximal response. With pipet mixing (green) the response decreases to 48 % maximal response. These results suggest plate mixing improves the concentration response curve to more closely reflect expected linear activity; by contrast pipet mixing resulted in higher activity (48%) than no mixing (35%) at lower concentrations tested. Analyzing the data with negative control correction increased the response similar to pipet mixing.

Summary:

1. **Plate mixing** reduced the DMSO addition artifact.
2. **Pipet mixing** resulted in signal oscillations over the DMSO addition artifact.
3. **Plate mixing** decreased the relative response to lower hyperpolarizing reagent concentrations as compared to pipet mixing.
4. **Plate mixing** decreased the relative response to KCl at 17 mM as compared to **pipet mixing**.

Fluidic Mixing Affects the Kinetic Response to KCl Mediated Depolarization

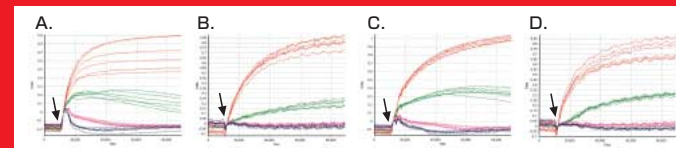


Figure 4. Three concentrations of KCl (Red =50 mM, Green = 17 mM, Purple = 5.6 mM) plus DMSO-only (Blue) were assayed with the following hardware parameters: (A) Cell plate mixing OFF and pipet mixing OFF (B) Cell plate mixing ON and pipet mixing OFF (C) Cell plate mixing OFF and pipet mixing ON (D) Cell plate mixing ON and pipet mixing ON.

Note the DMSO artifact immediately after addition (A); the artifact could be a result of cellular depolarization or incomplete DMSO mixing. In any case with plate mixing on the DMSO artifact disappears (B). With pipet mixing the DMSO artifact, along with a pipet mixing artifact (a dip in signal at 15,000 ms) is present (C). Pipet mixing plus cell plate mixing (D) gives results similar to panel B.



Figure 5. Data from Figure 4 (A) presented with negative control correction. Data presented as the mean±SD of six replicates.

Figure 6. The net changes (DMSO-only subtraction) of the three KCl concentrations were calculated from data in Figures 4 and 5. The maximal response (using 50 mM KCl) for each group was set at 100%; concentrations 17 and 5.6 mM are reported as percent activity of 50 mM. Plate mixing decreased the response at 17 mM as compared to no mixing. Neither pipet mixing nor negative control correction had any effect on the response as compared to no mixing.

References:

1. Dreesen J., Gentsch J., Graber N. Rapid Homogenization of Single Step Dilutions in Miniaturized 1,536 MTP Liquid Handling. Soc. Biomol. Screen. Conf. 2004 Poster P08011.

