Comparison of No Wash Reagent Kits on the FDSS6000

Introduction

Several commercially available 'No-Wash' reagent kits for measuring calcium mobilization using the FDSS6000 are now available. In some cases materials contained in a given kit may affect assay performance. Each kit offers advantages in terms of cost, performance, ease of use, availability, technical support, etc.
We present examples showing the effect of kit choice on assay performance, in two separate studies.

Study 1:

Comparison of BD™ PBS Calcium Assay Kit (BD Biosciences) **versus No Wash Kit X.**

Materials and Methods

Two kits were used in the study, BD™ PBS Calcium Assay Kit (BD Biosciences / www.bdbiosciences.com) and No Wash Kit X. Directions for use were followed per instructions. Assays were run on the FDSS6000, 384 well configuration, with four Xenon Lamps. The same agonist concentration was used against both kits. The data is expressed as the change in Fluorescent Counts subtracting the raw counts at reading 10 using CeuticalSoft (Hudson, NY). For each group n=16.

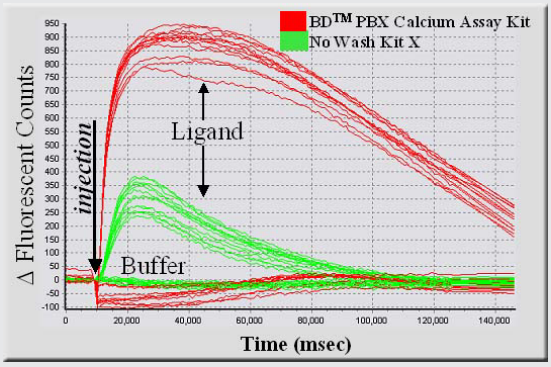

Fig. 1: Traces showing the response to agonist with the indicated kits. Note the response to the same agonist concentration is higher and longer with the BD kit as compared to X kit.

The Z' factor for the BD™ PBS Calcium Assay Kit was 0.76 with average starting counts of approximately 1900. The Z' factor for Kit X was 0.41 with average starting counts of approximately 1300.

The results indicate that for this cell line and agonist the BD™ PBS Calcium Assay Kit delivers a greater dynamic range and nearly double Z' factor as compared to Kit X.

Results

A recombinant cell line expressing Receptor A was used in the study. Cells were agonized against Ligand 1 and Ligand 2 (Figure 2).

Cells show calcium mobilization (decrease in fluorescence at ex 380 nm) following exposure to both Ligand 1 and 2 (Figure 2(a)). Figure 2(b) shows Ligand 1 induces membrane potential hyperpolarization while Ligand 2 induces depolarization.

Study 2:

Comparison of Fluo-4 NW (Invitrogen (IVGN)) **kit versus No Wash Kit X.**

Materials and Methods

Two kits were used in the study, Fluo-4 NW (Invitrogen / www.invitrogen.com) and No Wash Kit X. Directions for use were followed per instructions. Assays were run on the FDSS6000, 96 well configuration, with two Xenon Lamps. The same agonist was used against the three cell lines. Using CeuticalSoft for data analysis (Hudson, NY) the pseudoratio with reading 10 as the denominator was calculated. The difference between the maximal and minimal values was calculated and used to build the EC_{50} curves (Graphpad, San Diego, CA). For each dilution n=4, error bars are SEM.

Results

Figure 1 presents the results using Cell Line A using two kits (indicated). No response to agonist was detected using No Wash Kit X.

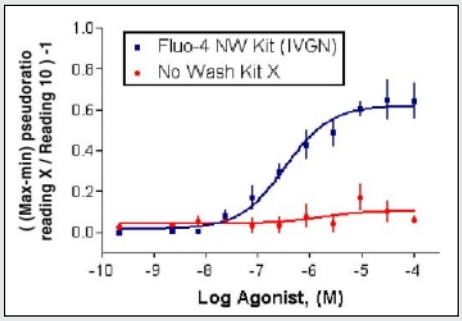

Fig. 1: Dose response curves of Cell Line A using two No Wash Kits. Note the use of No Wash Kit X results in an assay with no apparent efficacy to the agonist.

In Figure 2, using Cell Line B, both kits show equivalent efficacy yet there is a 140 fold difference in EC_{50} potency.

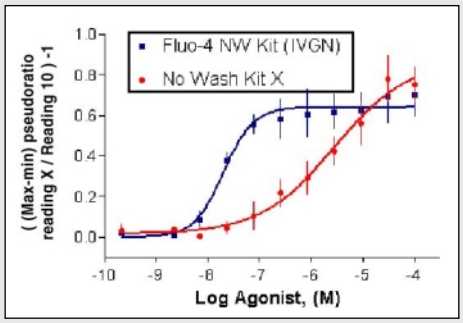

Fig. 2: Dose response curves of Cell Line B using two No Wash Kits. The efficacy of both kits is similar yet Fluo-4 NW kit has an EC_{50} 140 times more potent than No Wash Kit X.

Finally, results in Figure 3 show both kits exhibit nearly equivalent activity (Cell Line C).

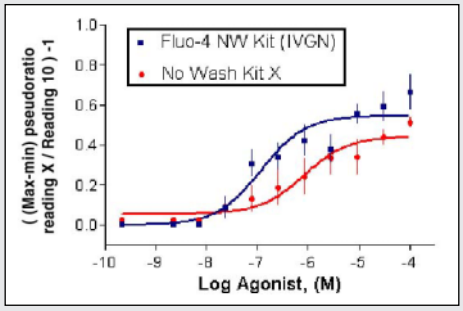

Fig. 3: Dose response curves of Cell Line C using two No Wash Kits. The efficacy of both kits is similar, EC_{50} of Fluo4NW is 7 times more potent than No Wash Kit X.

Conclusion

The wide disparity in assay performance using No Wash Kits suggests a complete strategy for assay development should include testing all available kits against all cell lines and agonists considered.

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