

Improved FLIPR HTS for a Chemokine GPCR Using MDCs Calcium Plus Reagent

Dr. Ralph Garippa, Hoffmann-La Roche, Nutley New Jersey USA

We have successfully completed a high throughput screening campaign on the FLIPR 384 system using the recently introduced Calcium Plus dye from Molecular Devices. The particular GPCR target addressed was problematic in assay development, owing in large part to its nature as a chemokine receptor. Our data shows that, in comparison to Fluo-4 and MDC's standard No Wash dye, the Calcium Plus reagent gave superior performance. The indices measured which demonstrated improvement include higher signal amplitude, fewer false positives, and improved inter/intra plate variability. The enhanced signal effect was specific to this chemokine GPCR as three other unrelated GPCRs were tested across all three dyes and no technical advantage was observed. Characterization with a known agonist/antagonist pair revealed EC50 and IC50 values that were consistent with literature reports. With stable expression of this GPCR in CHO-K1 Ga16 cells, we were able to identify several chemical classes of compounds with antagonistic activity specific to the receptor. In conclusion, use of the Calcium Plus dye was enabling for the FLIPR-based HTS of this chemokine GPCR.