Combining FLIPR and Transfluor: A Novel Assay for the Sequential Analysis of Calcium Flux and Receptor Desensitization


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Abstract
Two of the most widely used cell-based techniques for GPCR function are:
1) monitoring intracellular calcium with fluorescent dyes using FLIPR® and
2) image analysis of beta arrestin-mediated GPCR desensitization using the Transfluor® assay. Since the studies of these signaling pathways are complementary, we have developed a novel, sequential and homogeneous assay for FLIPR and Transfluor analysis on the same cells. A novel calcium dye (red emission) and FLIPR are used in combination with a Transfluor-enabled cell line and a new imager to analyze calcium signaling and receptor desensitization in the same cells. Experiments with the Angiotensin receptor demonstrate the compatibility and robustness of the two assays and the equivalent pharmacology as measured independently. In addition, the synergy between the two analysis technologies, with respect to the Angiotensin receptor, can be used to reveal ligands that signal and do not desensitize (NDLs) as well as inhibitors of the desensitization.

Introduction
GPCRs are proven targets in a wide range of major disorders. The Human Genome Project identified over 1,000 GPCRs, 200 of which are orphan GPCRs whose ligands have yet to be identified. The physiological relevance of cell-based assays has made them a crucial tool for the study of G-protein-coupled receptors (GPCR) function. Molecular Devices offers complimentary tools for GPCR drug discovery:
- FLIPR allows the monitoring of intracellular calcium with fluorescent dyes.
- Transfluor is an image-based assay for the beta arrestin-mediated GPCR desensitization.
- Curve Fitting - Hill, EC50

Figure 1: Redistribution of arrestin-GFP

Figure 2: Transfluor Assay

Materials and Methods
2. Add Can++ Red Dye (MDC’s Prototype kit) with FlexStation (96 wells).
3. Read Can++ with FLIPR.
4. Add fixative and nuclear dye.
6. AcuityXpress, MetaXpress and ImageXpress™ are trademarks and FLIPR, FlexStation, SoftMax and Transfluor are registered trademarks of Molecular Devices. All other trademarks are the property of their respective owners.

Conclusion
FLIPR reagents are compatible with Transfluor. GPCR doesn’t significantly interfere with Prototype Calcium Red Kit.
Equivalent performance of Calcium flux and Transfluor assay
- Transfluor gives excellent results at 8 minutes with the Angiotensin receptor (data not shown).
Software development of Transfluor HT Application Module
- Less than 15 minutes/ 384 well plate.
FLIPR followed by Transfluor is ideal for pathway mapping
- Compare cell signaling and beta-arrestin mediated desensitization.
- Revealed ligands that signal and do not desensitize (NDLs) as well as inhibitors of the desensitization mechanisms (DACs).