Application of multiplex cAMP and label-free assays using FDSS7000 and EPIC BT for studying potential biased signaling through the 5-HT$_7$ receptor

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Accelerate drug discovery

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Introduction

Agonist biased signaling by G protein coupled receptors (GPCRs) has been implicated in drug discovery and aided by multiple cell-based screening assay formats. The serotonin 5-HT$_7$ receptors are highly expressed in brain regions important for learning and memory processes and are known to couple to Gsα proteins. The potential for coupling to alternative signaling pathways has not been explored on 5-HT$_7$ receptors using label free technologies.

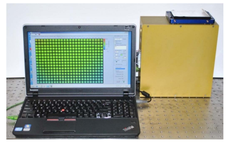
The present study was aimed to investigate the utility of cAMP and label-free assays for measuring potential biased signaling by the 5-HT$_7$ receptor (isoform b) stably expressed in HEK293 cells.

The accumulation of cAMP was measured both by a cell lysis-based end-point cAMP assay and a kinetic real-time fluorescence-based assay using membrane potential dyes on a Hamamatsu Functional Drug Screening System 7000 (FDSS7000) instrument in a 384 well format. The fluorescent membrane potential readout was achieved through co-expression of modified cyclic nucleotide-gated (CNG) channels (ACTOne), co-localized with adenylate cyclase at the plasma membrane, as a biosensor of cAMP levels. Our label-free assay was conducted with a Corning EPIC BT system, which measures cellular dynamic mass redistribution (DMR) using resonant waveguide grating (RWG) biosensors embedded in the 384-well assay plates.

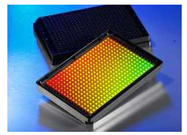
Materials and Methods

- ACTOne parental HEK293 cells with stable expression of the CNG channels; ACTOne cells stably expressing the human 5-HT$_7$ receptor; ACTOne cells stably expressing the human thyroid stimulating hormone receptor (TSHR)
- Endogenous beta-adrenergic and adenosine A2b receptors in ACTOne cells were assessed with isoproterenol and NECA
- Corning Fibronectin-coated Epic 384 plates; Corning Costar poly-D-lysine (PDL)-coated 384 black/clear plates; ACTOne Membrane Potential Dye; Cisbio HiRange cAMP HTRF assay kit
- Special calibration of the Hamamatsu FDSS 7000 instrument for the EPIC optical plate includes the introduction of a new assay plate type, as well as the creation of plate map, autofluorescence and shading correction files.

FDSS 7000



EPIC BT



EPIC 384w Microplate

Results and Conclusions

- **Cellular responses mediated through the G$_s$-coupled 5-HT$_7$ receptor in ACTOne cells were measured in 3 assay platforms: cAMP HTRF, membrane potential and Epic label-free.**
- **A negative DMR was measured in ACTOne-5HT$_7$ cells with 5-HT and 8-OH-DPAT. The response magnitude and potency with endogenous receptor activation (G$_s$-coupled) were reduced in ACTOne-5-HT$_7$ cells compared with parental cell line.**
- **A unique integrated assay procedure for measuring cellular cAMP and DMR phenotypic responses on FDSS and EPIC platforms was established, though in pre-selected ACTOne cells stably expressing G$_s$-coupled receptors seem to have reduced cAMP responses.**
- **Data from the limited set of 5-HT$_7$ compounds suggest a larger number of compounds may be needed to study potential biased signaling through the 5-HT$_7$ receptor**

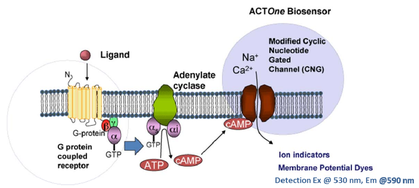
Table 1. Summary of ACTOnce-5-HT$_7$ EC$_{50}$ and IC$_{50}$ data from three assay platforms

EC$_{50}$ (nM)	Membrane potential (FDSS)	DMR (Epic)	cAMP (HTRF)	IC$_{50}$ (nM)	Membrane potential (FDSS)
5-HT	0.5	88	3601	SB-269570	5.1
8-OH-DPAT	101	69	4816	SB-258719	259
LP 44	3487	>10,000	>10,000		
Isoproterenol	6854 **(0.4)**	>10,000 **(14)**	>10,000 **(82)**		
ATP	>10,000	659 **(544)**	>10,000		

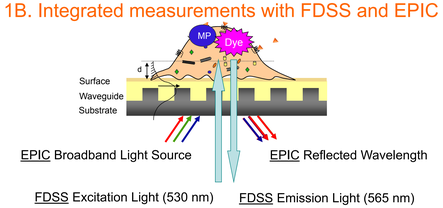
Results from ACTOne parental cells are either >10,000 nM or with no response, **unless indicated in parenthesis**

Figure 1. An integrated assay procedure for measuring cellular responses on FDSS and EPIC platforms using a single set of cell (Epic) and compound plates

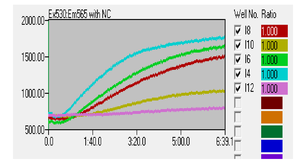
1A. Measuring cAMP with ACTOne on FDSS



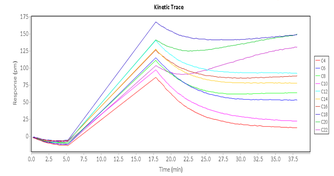
1B. Integrated measurements with FDSS and EPIC



1C. cAMP kinetic traces on FDSS



1D. cAMP kinetic traces on Epic

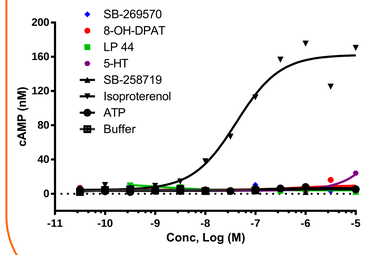


Steps	Cell seeding in EPIC plates (15K, 30 ul)	Incubation	Cell wash*	Add MP dye (30 ul)	▪ Equilibration ▪ Dye loading	EPIC baseline DMR reading	▪ Compound addition for both FDSS and EPIC ▪ Membrane potential kinetic reading on FDSS	DMR-response reading on EPIC
Time	5 min	Overnight	3 min	1 min	2 hour	3 min	5-15 min kinetic or endpoint (RT)	15-60 min kinetic or endpoint (RT)

* *Cells in EPIC plates were washed with assay buffer containing HBSS, 0.05% BSA, 0.5-1% DMSO using a Biotek ELX50 strip washer;*

Figure 2. cAMP measurement using Cisbio HiRange cAMP assay kit

2A. ACTOne parental cells



2B. ACTOne-5-HT$_7$ cells

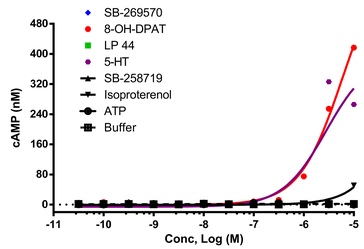
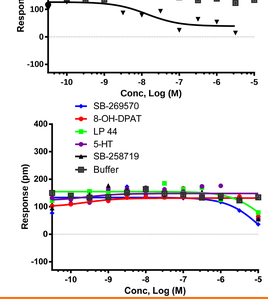


Figure 3. Phenotypic measurement of DMR responses on EPIC BT

3A. ACTOne parental cells



3B. ACTOne-5-HT$_7$ cells

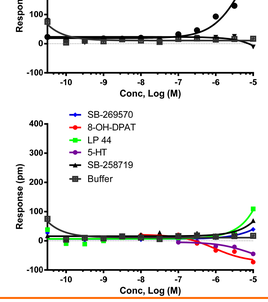
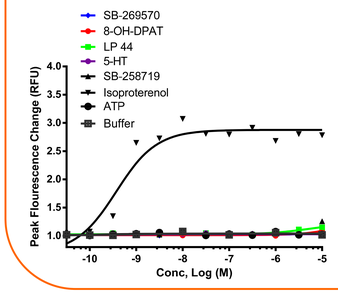


Figure 4. cAMP measurement using membrane potential dye on FDSS

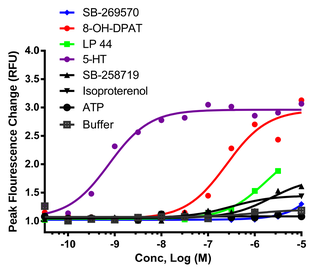
- ACTOne biosensor is selective for G$_s$ pathway - no response with ATP

4A. ACTOne parental cells



4B. ACTOne-5-HT$_7$ cells

Agonist



Antagonist

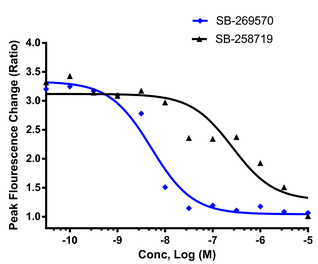
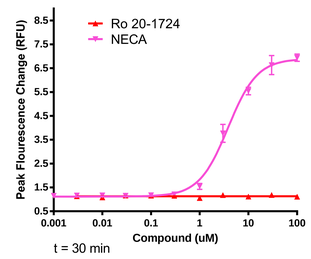


Figure 5. ACTOne cells with stable expression of G$_s$-coupled receptors has reduced cAMP responses by endogenous receptors using MP dye on FDSS

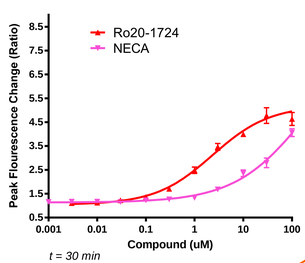
- ACTOne-TSHR cells has elevated basal cyclase activity, as seen with PDE inhibitor Ro 20-1724

5A. ACTOne parental cells



t = 30 min

5B. ACTOne-TSHR cells



t = 30 min