Cellomics™

Shifting Discovery into High Gear

GPCR Signaling BioApplication

Catalog No. S50-0013-2

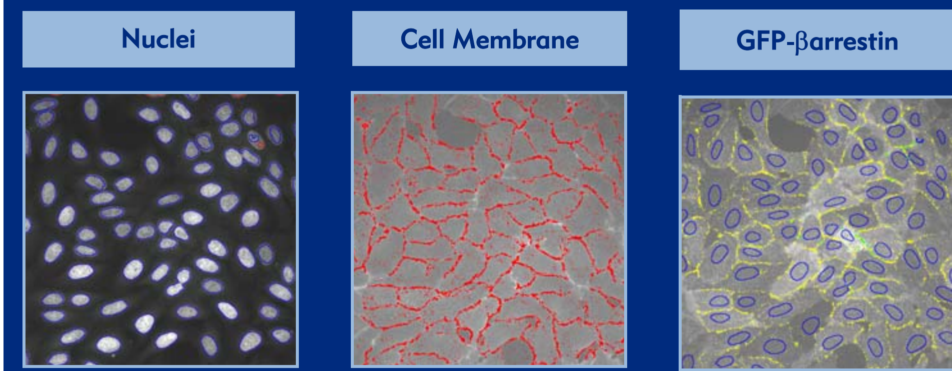

Figure 1. Demonstration of the GPCR Signaling BioApplication utilizing Transfluor® technology. Cells expressing both GFP-βarrestin (right) and the β2-adrenergic receptor were stained with Hoechst nuclear dye (left) and a red fluorescent cell membrane marker (center). Images of all three fluorescent channels were automatically acquired on an ArrayScan® HCS Reader and analyzed using the GPCR Signaling BioApplication. Nuclei (blue overlays) and membranes (red spots) are identified automatically, and the distribution of the GFP-βarrestin fusion (yellow and green spots) is determined. In this example of early activation the receptor is mostly in small pits co-localized with the membrane.

Description

Approximately three-quarters of today's drugs act upon some type of GPCR. A major focus of drug discovery research is finding more effective drugs that act upon these targets, as well as characterizing new "orphan" receptors and validating them as drug targets. With the GPCR Signaling BioApplication from Cellomics, Inc., these types of studies can be streamlined to gain critical information more rapidly than ever before.

This BioApplication utilizes three fluorescent probes for image analysis: nuclear, cell membrane, and target molecule (for example GFP-βarrestin). The application is able to detect and classify the different sub-cellular distribution phenotypes for βarrestin: diffuse cytoplasmic, cell membrane, clathrin-coated pits, and endosomes. The GPCR Signaling BioApplication can distinguish among any of these phenotypes, making it universally applicable to receptors of different classes and types.

Norak Biosciences' Transfluor® technology utilizes the steps of GPCR internalization and desensitization – common to almost all GPCRs – to create a robust assay for GPCR activation. By labeling ßarrestin with GFP, one can classify and characterize these processes by high content screening. The GFP-ßarrestin is evenly distributed throughout the cytoplasm in the absence of receptor activation. In response to stimulation, the translocation of ßarrestin to the membrane is very robust, as evidenced by the co-localization of the ßarrestin (yellow overlay, Figure 1, right) with the cell membrane marker (red overlay, Figure 1, center). The BioApplication is able to automatically detect and quantitate these changes in subcellular distribution. The BioApplication reports the percentage of cells at each dose of agonist that display the activated phenotype.

We have successfully performed a high content screen for activation of the ß2-adrenergic receptor with Transfluor technology using this BioApplication. The quality of this assay was determined through use of a

Features

- Detects and quantifies activation of both Class A and Class B receptors
- Universal for any GPCR that internalizes
- Automatically classifies phenotype based on subcellular target molecule distribution (e.g. cell membrane, internalized clathrin coated pits, receptor-containing endocytic vesicles)
- Characterizes cell populations and allows correlation with other features of interest

Benefits

- Rapid start-up and assay development via turnkey image analysis module
- Better, higher-content information with multiple quantitative outputs
- Combination of platform and Transfluor technology provides comprehensive solution for GPCR drug discovery

Z' window analysis; half of triplicate microplates were treated with medium only or maximal agonist stimulation (370 nM isoproterenol). Analysis of these “Min-Max” plates indicated exceptional performance with a Z′ window of 0.73. This value, along with high signal-to-noise (S/N) and signal-to-background (S/B) ratios (Table, right) indicate that the GPCR Signaling BioApplication provides a robust analysis of GPCR activation and βarrestin redistribution.

Assay Performance	Mean	SD
Magnification	20x	-
Z′ factor	0.73	.03
Signal/Noise	15.7	1.7
Signal/Background	6.5	0.6
EC50 (nM)	10.8	1.7

In addition to screening, we have examined a number of β2-adrenergic receptor agonists and antagonists. For antagonist experiments, cells were pre-incubated with antagonist for 20 minutes followed by stimulation with agonist for seven minutes. As shown in Figure 2, the GPCR Signaling BioApplication is able to detect and quantify antagonism of cimeterol, a weak agonist, by various compounds. Detailed information about potency, and percentage of cells that respond to each agonist or antagonist provide a better understanding of the receptor’s biology, enabling one to distinguish, for example, partial agonists from full agonists.

The ß2-adrenergic receptor represents a prevalent sub-class of GPCRs in which arrestins dissociate from the receptors near the cell membrane after activation. In class B GPCRs, βarrestins remain associated and translocate with the receptors into endocytic vesicles. In the latter case, the GFP-ßarrestin forms large punctate spots in the perinuclear region. The Cellomics platform with Transfluor technology is able to successfully detect and classify the distribution of GFP-ßarrestin in all stages of redistribution, thereby providing the

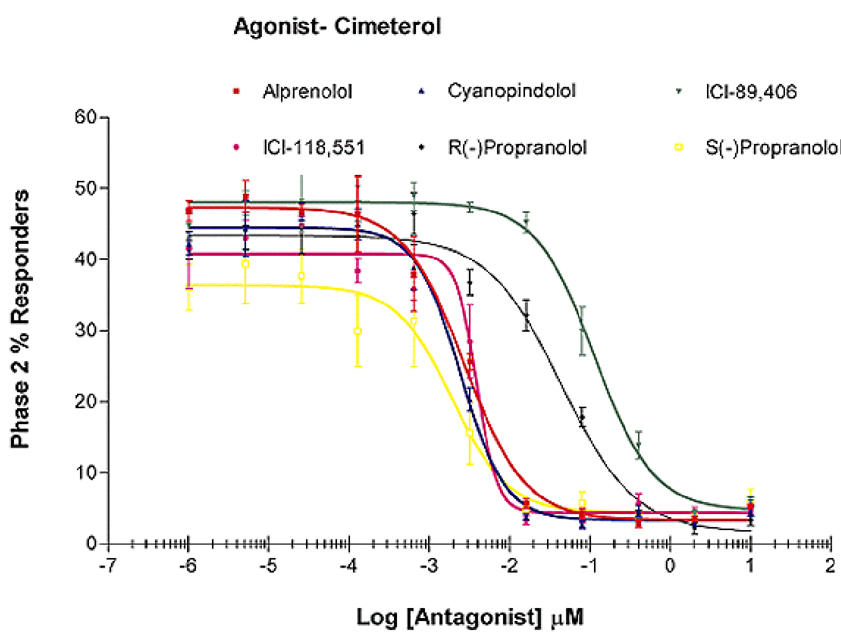

Figure 2. Dose response curves for antagonism of various compounds against cimeterol mediated β_2AR activation in U2OS cells. Cells were treated with different compounds as described above. Data is mean ± sem from at least 4 wells per concentration.

Cellomics, Inc. is pioneering High Content Screening (HCS) to automate information-rich biological assays for the discovery and validation of new drugs. HCS analyzes multiple interacting or independent targets in intact cells simultaneously, using state-of-the-art fluorescent reagents, cells, advanced optical imaging instrumentation and both informatics and bioinformatics tools. Through HCS, drug screening is based upon target activity, location, and kinesis, as well as interacting cellular components and pathways, morphological events, and environmental factors that combine to elicit a biologically relevant whole cell response.

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