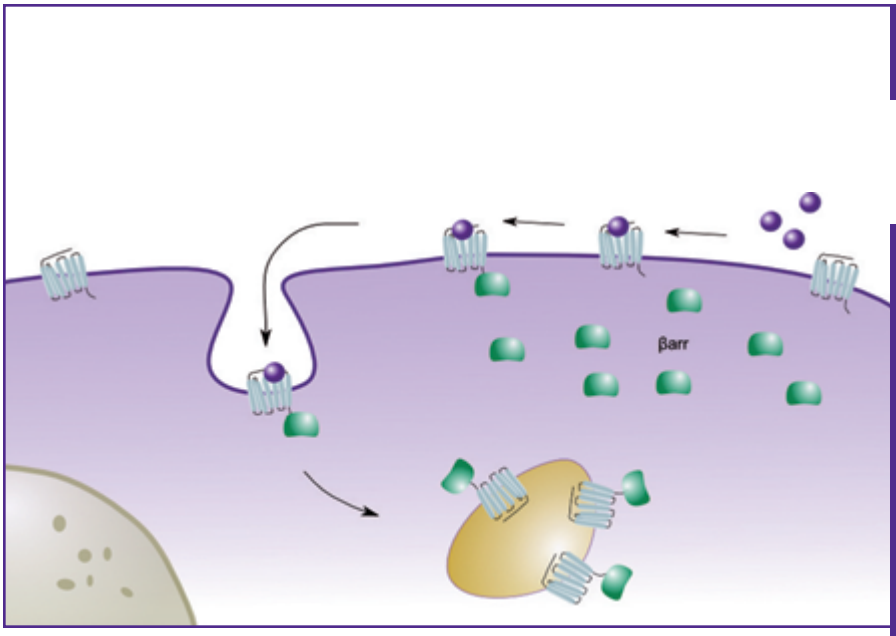

APPLICATION NOTE

A functional assay for the Beta 2-Adrenoreceptor (β2AR) using the Opera

Key Features

- Confocal image acquisition of live cells using the Opera™ High Content Screening system
- Image analysis using the Acapella™ Spot Detection software
- Quantification of pit formation upon activation of the β2-adrenergic receptor

Spot detection, GPCR, clathrin-coated pits

Background

The beta adrenergic receptor (βAR) is one of the most important targets for the treatment of hypertension and heart failure. Screening for βAR modulators can be achieved using Transfluor® technology. This assay principle makes use of the recruitment of β-arrestin molecules to activated receptors and their traffic within the cell and is universally applicable to GPCR activation. Since the readout is a translocation event from a homogeneous distribution of the β-arrestin molecules within the cytoplasm to clathrin-coated pits and endosomes, it requires a high resolution imaging system with quantitative image analysis capability.

Application

The Opera is the ideal platform to perform the Transfluor® Pit Forming Assay, because its confocal imaging capability results in very high data quality. β-arrestin redistribution can be assayed by monitoring and quantifying the fluorescence of a GFP fusion protein genetically engineered with β-arrestin. Both agonistic and antagonistic assay scenarios are feasible. The assay signals can be normalized and averaged to the number of cells in the assay by identifying and locating individual cells with nuclear dyes.

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The β-arrestin and nuclear signals are imaged simultaneously by employing both Opera's CCD cameras. In order to run the Transfluor® Pit Forming Assay on the Opera, a variety of options for the assay set-up can be chosen. Different plate types and objectives with different magnification and numerical aperture can be used. Also different plate formats such as conventional 96-, 384- or 1536- well plates are compatible with this assay. Depending on the plate format, throughputs of up to 80,000 data points per day are feasible. Reliable imaging conditions ensure the robustness required for a high-throughput screening (HTS).

Conclusions

By evaluating the Transfluor® Pit Forming Assay with the Acapella Spot Detection algorithm a detailed quantification of pit formation upon activation of the 2-adrenergic receptor is possible. This meets the need for assay development as well as high throughput screening. EC_{50} and IC_{50} values can be determined with high accuracy.

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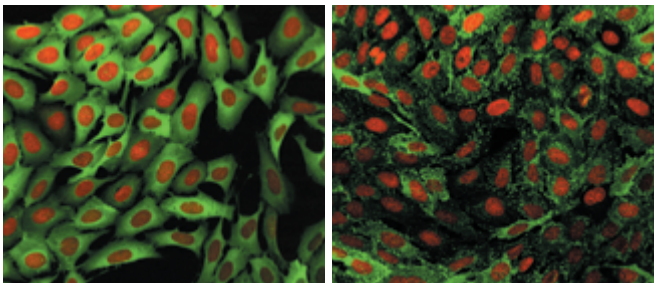

Figure 1: Opera images of the Transfluor® Pit Forming Assay using a 20X NA 0.7 water immersion objective. In non-stimulated cells, βarr2-GFP molecules display a homogeneous distribution within the cytoplasm *(left image)*. Following incubation with agonist, βarr2-GFP internalizes with the beta adreno-receptor (βAR) into clathrin-coated pits *(right image)*. Nuclei are visualized by propidium iodide after fixing the samples.

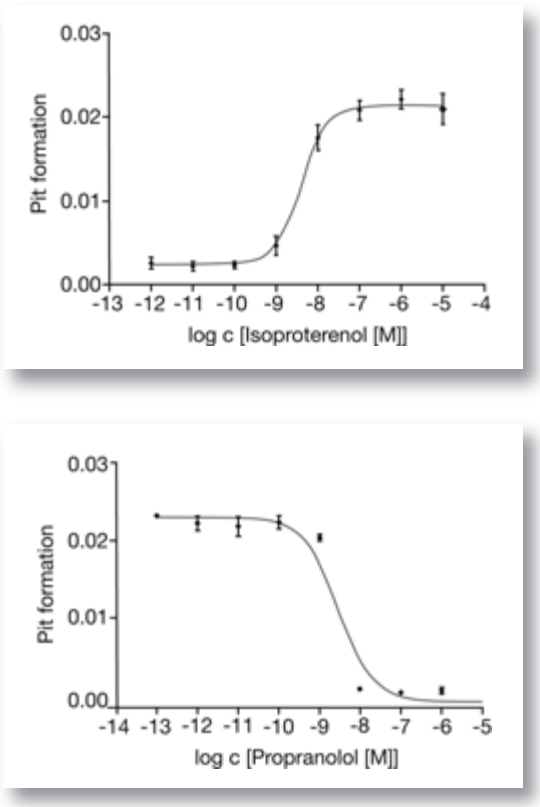

Figure 2: The graphs show normalized pit formation of dose-dependent beta adreno-receptor activation/inhibition with respective ligands. The *top* curve shows EC_{50} determination with Isoproterenol resulting in an EC_{50} value of 3.89 nM. The *bottom* curve shows inhibition of Isoproterenol binding with Propranolol in a dose-dependent fashion resulting in an IC_{50} value of 2.93 nM. The signal-to-background ratio in this assay was 22.5 and the Z' value calculated from 24 positive and negative samples was calculated to 0.76.

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