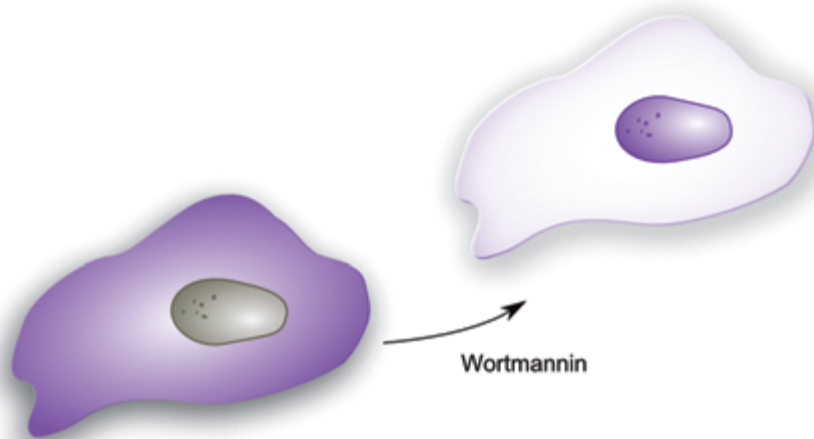


Analysis of the Forkhead Redistribution[®] Assay using the Opera

Key Features

- Confocal image acquisition of live cells using the Opera™ High Content Screening system
- Image analysis using the Acapella™ Nuclear Translocation algorithm software
- Detection of the cytoplasm and nuclear area



Nuclear import, FKHR translocation

Background

Transcription factors such as FKHR function as key regulators in e.g. insulin signalling, cell cycle progression and apoptosis downstream of phosphoinositide 3-kinase (PI3K). Inactive FKHR is cytoplasmic but is rapidly imported to the nucleus upon inactivation of the PI3K/Akt pathway. The Forkhead Redistribution[®] Assay is designed to assay for inducers of FKHR translocation in FKHR-GFP fusion protein expressing cells. Monitoring the translocation of the fusion protein from the cytosol to the nucleus requires a high resolution imaging technique.

Application

The Opera is the ideal platform to perform the Forkhead Redistribution[®] Assay because its confocal imaging capability leads to very high data quality. FKHR translocation can be assayed by monitoring and quantifying the fluorescence of a FKHR-GFP fusion protein in the cytosolic and the nuclear cellular compartment. Compounds causing nuclear accumulation of FKHR could be directly interfering with the PI3K/Akt1 pathway, or could be general nuclear import activators or nuclear export inhibitors.

In order to run the Forkhead Redistribution® Assay on the Opera, a variety of options for the assay set-up can be chosen. Different plate types and lenses with different magnification and numerical aperture can be used. Also, different plate formats such as 96- or 384-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) situation.

The Forkhead Redistribution® Assay is evaluated using an Acapella™ Nuclear Translocation algorithm software, enabling detailed cytoplasmic and nuclear area detection.

Conclusions

In this study EC_{50} values can be determined with high accuracy by using a robust cell-based assay with the Acapella High Content Analysis software.

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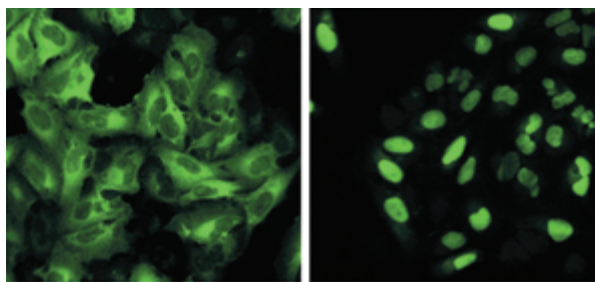


Figure 1: Opera images of the Forkhead Redistribution® Assay using a 20X NA 0.7 W water immersion objective. In non-stimulated cells, FKHR-GFP is located in the cytoplasm (left image). Following incubation with e.g. a PI3K inhibitor such as wortmannin, FKHR-GFP is accumulated in the nucleus (right image).

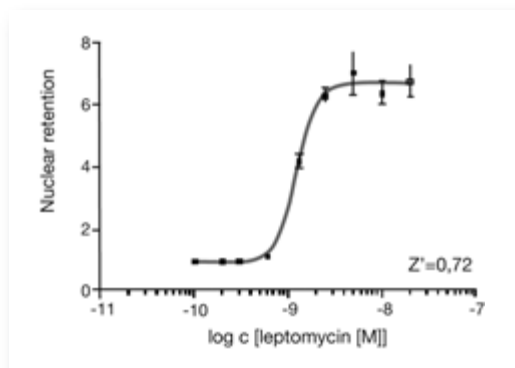
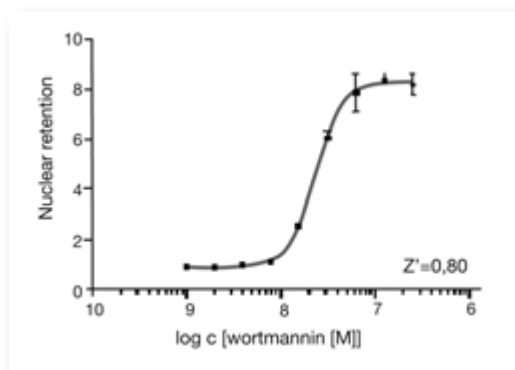


Figure 2: The graphs show normalized nuclear retention of dose-dependent PI3K inhibition using the Acapella Nuclear Translocation algorithm. The top curve shows EC_{50} determination with wortmannin resulting in an EC_{50} value of 23.6 nM. The bottom curve shows EC_{50} determination with leptomycin resulting in an EC_{50} value of 1.23 nM. The Z' value for the Forkhead Redistribution® Assay was calculated to 0.72-0.80.