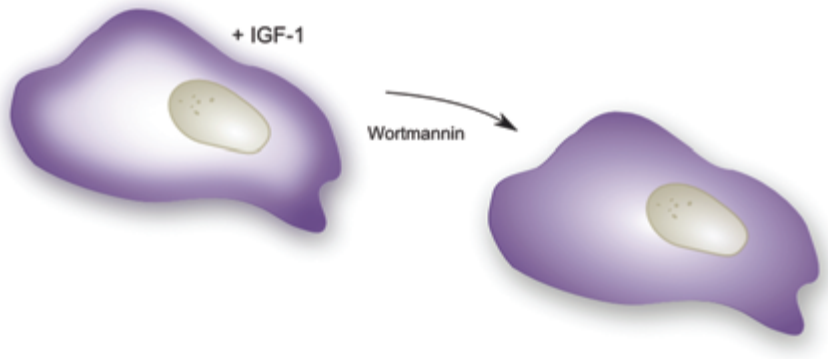


APPLICATION NOTE

The Btk-PH Domain Redistribution® Assay using the Opera

Key Features

- Automated confocal image acquisition of fixed cells using the Opera™ High Content Screening system
- Image analysis using the Acapella™ Membrane-to-Cytosol Translocation algorithm
- Quantification of Btk translocation

Membrane-to-cytosol translocation, IGF-1, wortmannin

Background

Bruton's tyrosine kinase (Btk) is a cytoplasmic protein tyrosine kinase (PTK) crucial for B-cell development and differentiation. It binds to phosphatidylinositol-3,4,5- trísphosphate (PIP3) through the Btk pleckstrin homology (PH) domain. Upon activation, Btk translocates from the cytoplasm to the plasma membrane due to the production of PIP3 by phosphatidylinositol 3-kinase (PI3K) in the membrane. For monitoring the translocation of a Btk-PH-GFP fusion protein from the cytoplasm to the plasma membrane, a high resolution confocal imaging technique and a sophisticated algorithm for quantification of this event is required.

Application

The Opera is the ideal platform to perform the Btk-PH Domain Redistribution® Assay, because its confocal imaging capability leads to very high data quality. Btk translocation can be assayed by monitoring and quantifying the fluorescence of a Btk-PH-GFP fusion protein in the cytosolic compartment and the plasma membrane. Using Insulin-like growth factor-1 (IGF-1) as a reference agonist, compounds can be assayed for their ability to inhibit IGF-1-stimulated membrane translocation of Btk-PH. Inhibiting compounds could be interfering directly with Btk-PH translocation or they could act upstream of Btk.

PerkinElmer®
For the Better

In order to run the Btk-PH Domain Redistribution® Assay on the Opera, a variety of options for the assay set-up can be chosen. A range of plate types and objectives 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. Dependent 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 Btk-PH Domain Redistribution® Assay is evaluated using an Acapella Membrane-to-Cytosol Translocation algorithm which is especially powerful in detecting gradual changes between activated and non-activated stages of translocation.

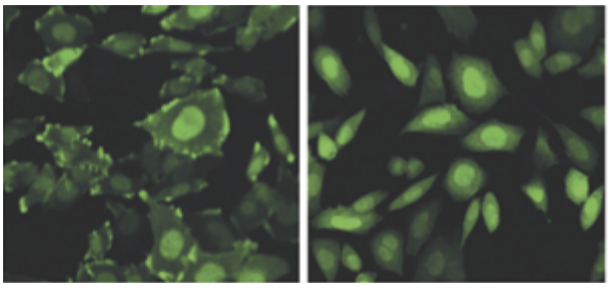


Figure 1: Opera images of the Btk-PH Domain Redistribution® Assay using a 20X NA 0.7 W water immersion objectives. In stimulated cells, Btk-PH-GFP is located in the plasma membrane cytoplasm *(left image)*. Following incubation with e.g. a PI3K inhibitor such as wortmannin, Btk-PH-GFP is retained in the cytoplasmic compartment *(right image)*.

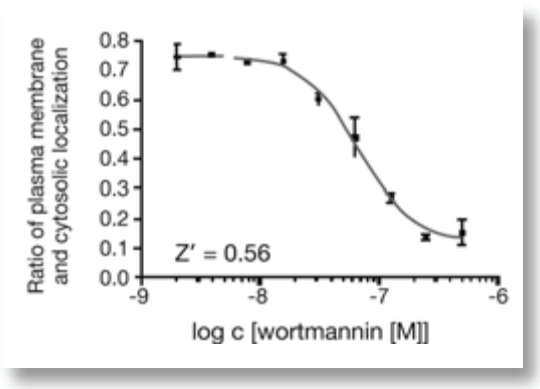
Conclusions

We demonstrate here that our Membrane-to-Cytosol Translocation algorithm is a robust and reliable tool for quantification of the Btk-PH Redistribution Assay. The Acapella software meets the need for assay development as well as high throughput screening. IC_{50} values can be determined with high accuracy.

Authors

Gabriele Gradl
Olavi Ollikainen

PerkinElmer
Cellular Technologies Germany GmbH
Cellular Imaging & Analysis
Hamburg, DE



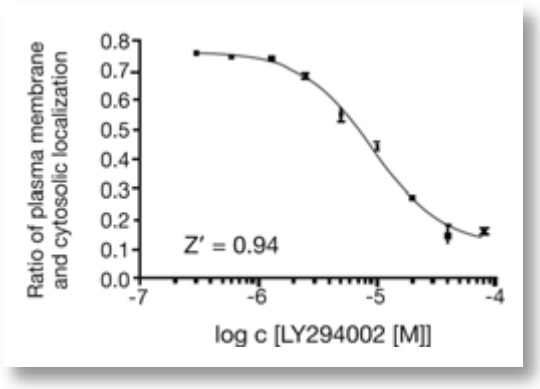


Figure 2: The graphs show the ratio of Btk-PH-GFP plasma membrane and cytosolic localization of dose-dependent PI3K inhibition using the Acapella Membrane-to-Cytosol Translocation algorithm. The *top* curve shows IC_{50} determination with wortmannin resulting in an IC_{50} value of 67.2 nM. The *bottom* curve shows IC_{50} determination LY294002 with resulting in an IC_{50} value of 9.2 µM. The Z' value for the Btk Redistribution® Assay was calculated to 0.56-0.94.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

008182A_01 Jan. 2010