

Thermo Scientific Btk-PH Redistribution[®] Assay

The Redistribution technology monitors the cellular translocation of GFP-tagged proteins in response to drug compounds or other stimuli and allows easy acquisition of multiple readouts from the same cell in a single assay run. In addition to the primary readout, high content assays provide supplementary information about cell morphology, compound fluorescence, and cellular toxicity.

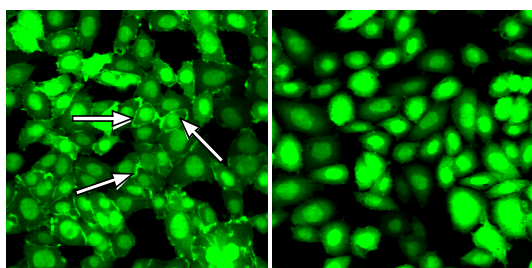


Figure 1. Membrane translocation of Btk-PH-EGFP. Cells were treated with 10 nM IGF-1 without (DMSO control, left panel) and with (right panel) addition of 500 nM wortmannin. Arrows indicate IGF-1 induced membrane translocation of EGFP-PDK1 detected by the image analysis algorithm.

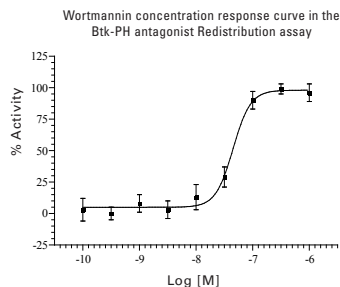


Figure 2. Concentration response curve in the Btk-PK antagonist assay. Wortmannin concentration response curve in the Btk-PH antagonist Redistribution assay stimulated by 100 nM IGF-1 (n=8). The EC₅₀ of wortmannin is 45 nM. Concentration response was measured in 9 point half log dilution series. Cells were incubated with 100 nM IGF-1 and wortmannin for 4 min. Cells were then fixed and membrane translocation was measured using the Cellomics ArrayScan V[™] Reader and the CytoCellMemTrans.V2 BioApplication. % activity was calculated relative to the positive (500 nM wortmannin) and negative control (0.25% DMSO).

Thermo Scientific Btk-PH Redistribution Assay

Bruton's tyrosine kinase (Btk) is a non-receptor cytoplasmic tyrosine kinase belonging to the Tec family of kinases. Btk mediates B lymphocyte proliferation, differentiation and signaling. Btk translocates to the plasma membrane via binding of its pleckstrin homology (PH) domain to phosphatidylinositol-3,4,5-triphosphate (PIP3) following growth factor stimulation and cross linking of the B cell receptor. Mutations in the Btk gene cause X-linked agammaglobulinemia (XLA). This disorder is characterized by lack of mature B cells resulting in primary immunodeficiency [1]. The Btk-PH Redistribution assay monitors the translocation of the Btk-PH-EGFP fusion protein from the cytoplasm to the plasma membrane. Insulin-like growth factor-I (IGF-I) is used as the reference agonist.

Test compounds are assayed for their ability to inhibit IGF-I-stimulated membrane translocation of the Btk-PH-EGFP

fusion protein. Wortmannin inhibits the phosphatidylinositol 3-kinase (PI3K) [2, 3] and hereby decreases the production of PIP3. Thus, wortmannin is used in the assay as reference antagonist. Test compounds inhibiting IGF-I-induced cytoplasm to membrane translocation of Btk may interfere directly with Btk-PH translocation or may act upstream of Btk. The Btk Redistribution assay can be used as a selectivity assay for the Akt Redistribution assays (see related products) to deselect or identify compounds working upstream of Akt at the level of PI3K.

Features

- Designed to assay compounds for their ability to modulate formation of PIP3 in the plasma membrane
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

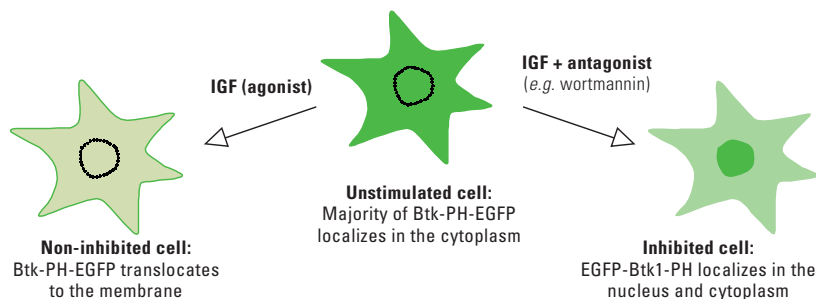


Figure 3. Illustration of the BTK-PH translocation event.

Highlights:

- **Biologically relevant data**
Compounds tested in a cellular environment
- **Validated**
Functionally tested cells provided with an optimized assay protocol
- **Easy to use**
Just plate cells, add compounds, and image

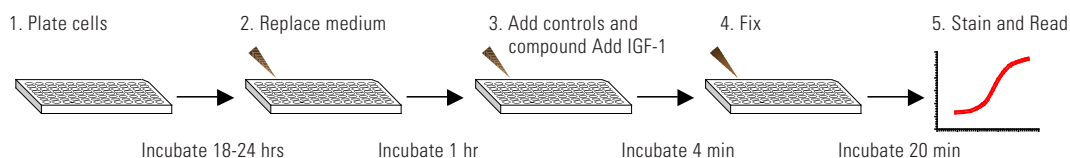


Figure 4. The Btk-PH Redistribution assay is very easy and fast to perform.

Thermo Scientific Btk-PH Redistribution® Assay

Assay Details

Recombinant CHOHR cells stably expressing human Btk-PH domain, Btk(1-180) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The Btk-PH assay is validated with an average $Z' = 0.49 \pm 0.05$, suitable for both screening and profiling applications.

Imaging

The translocation of Btk can be imaged on most HCS platforms and fluorescence microscopes. The filters should be set for Hoechst (350/461 nm) and GFP/FITC (488/509 nm) (wavelength for excitation and emission maxima). Consult the instrument manual for the correct filter settings. The translocation can typically be analyzed on images taken with a 10-20x objective or higher magnification. The primary

output in the Btk Redistribution assay is the translocation of Btk from cytoplasm to membrane spots. The data analysis should therefore report an output that corresponds to number, area, or intensity of these spots in the membrane.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC and the CytoCellMemTrans.V2 BioApplication. The output parameter used was MEAN_%MemColoc. The minimally acceptable number of cells used for image analysis in each well was set to 100 cells.

Other BioApplications that can be used for this assay include ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYORED1
007_01	Btk-PH Redistribution Assay	CHO	•	•	

The Redistribution Assays are available in 3 product formats, Profiling, Screening and CryoRedi, for different volume and level of convenience needs. The Redistribution Assays can also be accessed through the Thermo Scientific Managed Services.

Related Thermo Scientific Products

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYORED1
085_01	Akt1 Redistribution Assay	CHO	•	•	
011_02	Akt2 Redistribution Assay	CHO	•	•	•
012_01	Akt3 Redistribution Assay	CHO	•	•	
006_01	Akt1-PH Redistribution Assay	CHO	•	•	•
008_01	FKHR (FOXO1) Redistribution Assay	U2OS	•	•	•
009_02	FKHRL1 (FOXO3) Redistribution Assay	U2OS	•	•	•
090_01	AFX (FOXO4) Redistribution Assay	U2OS	•	•	
013_01	PDK1 Redistribution Assay	CHO	•		
8404101	Cellomics Phospho-AKT Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8407101	Cellomics Phospho-GSK-3 Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8403601	Cellomics Beta-Catenin Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
CX03004-INS	Cellomics ONE BioApplication Suite	High content data acquisition and analysis software			
CX03102A/B	Cellomics ArrayScan V ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. Rosen H & Goetzl EJ, *Nature Rev Immunol.*, 5, 560-570, 2005.
2. Kostenis E, *J Cell Biochem.*, 92, 923-936, 2004.
3. Okamoto H et al., *J Biol Chem.*, 273, 27104-27110, 1998.
4. Jo E et al., *Chem Biol.*, 12, 703-715, 2005.

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