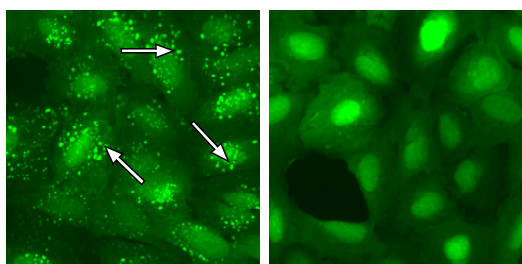


## Thermo Scientific FYVE Redistribution<sup>®</sup> Assay

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. Dissociation of EGFP-2xFYVE from early endosomes.** Cells were treated with 100 nM wortmannin for 30 min (right panel) or untreated (DMSO control, left panel). Arrows indicate the endosome-localized EGFP-2xFYVE detected by the image analysis algorithm.

### Thermo Scientific FYVE Redistribution Assay

Vesicular trafficking and proper sorting of internalized proteins require the recruitment of cytosolic proteins to a specific endosomal membrane in a reversible and regulated manner. Many proteins become reversibly localized to membranes through interaction with specific lipid head groups. PtdIns(3)P is a product of the class III PI3K Vps34 that is constitutively produced and specifically localizes to early endosomes. Several proteins bind specifically to the PtdIns(3)P head group through domains such as the FYVE-finger or the PX domain [1-5]. The FYVE-finger is a cysteine-rich double-zinc-binding domain, which is thought to localize FYVE-finger proteins to early endosomes through its interaction with PtdIns(3)P.

The FYVE domain Redistribution assay is designed to screen for inhibitors of FYVE endosomal localization by monitoring the dissociation of FYVE domains from early endosomal membranes. The EGFP-2xFYVE fusion protein used in this assay consists of

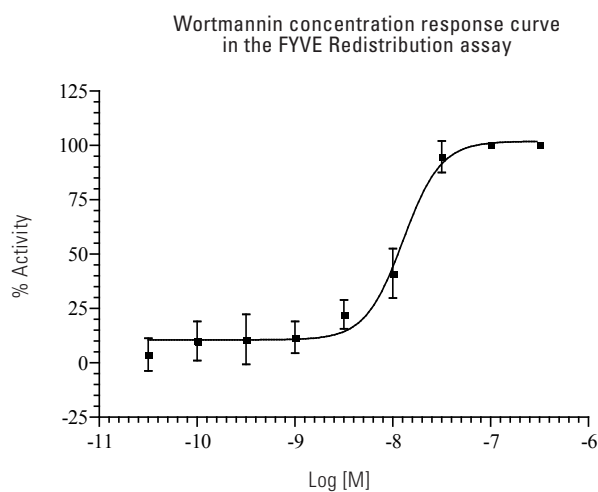
the FYVE finger from the human homologue of the hepatocyte growth factor-regulated tyrosine kinase substrate Hrs, duplicated in tandem. Hrs is involved in sorting of ubiquitinated proteins into clathrin-coated microdomains of early endosomes.

### Features

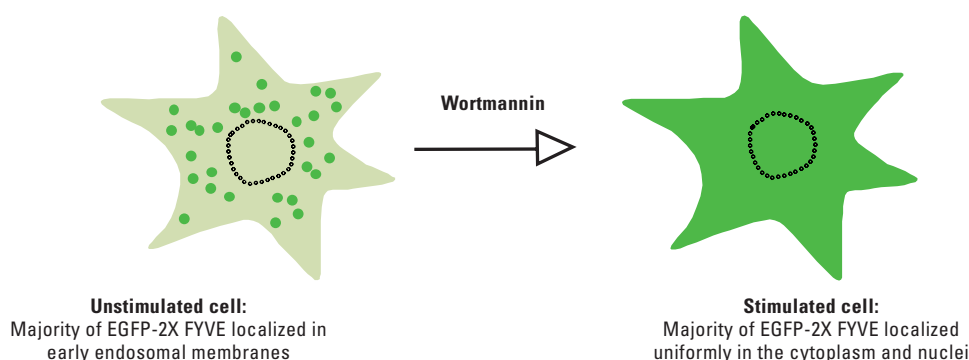
- Designed to assay compounds for their ability to dissociate FYVE from early endosomes
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

### 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 2. Wortmannin concentration response in the FYVE assay:** The  $EC_{50}$  of wortmannin is ~10 nM. Concentration response was measured in 9 point half log dilution series ( $n = 16$ ). Cells were treated with wortmannin for 30 min. Cells were then fixed and nuclear translocation was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (100 nM wortmannin) and negative control (0.25% DMSO)



**Figure 3.** Illustration of the FYVE translocation event.

## Thermo Scientific FYVE Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant U2OS cells stably expressing the FYVE finger from the human homologue of the hepatocyte growth factor-regulated tyrosine kinase substrate Hrs, duplicated in tandem and fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay monitors translocation of EGFP-2x FYVE from its initial location bound to (PtdIns(3)P) in early endosomes to the cytoplasm, in response to test compounds. FYVE translocation can be promoted by the PI3K inhibitor wortmannin [6, 7], which inhibits Vps34 catalytic activity and thereby prevents (PtdIns(3)P) synthesis. Wortmannin is used as reference compound in the assay. The FYVE assay is validated with an average  $Z' = 0.70 \pm 0.08$ , suitable for both screening and profiling applications.

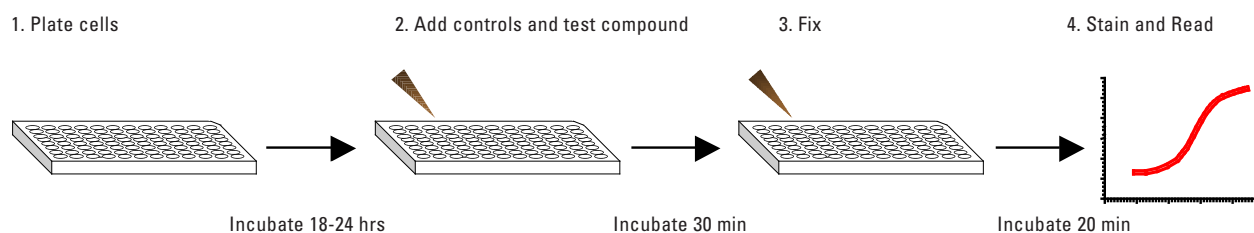
### Imaging

The translocation of EGFP-2x FYVE 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 10x objective or higher magnification. The primary output in the FYVE Redistribution assay is the dissociation of spots in the cytoplasm. The data analysis should therefore report an output that corresponds to number, area, or intensity of spots in the cytoplasm.

### Imaging on Thermo Scientific Cellomics ArrayScan V<sup>TI</sup>

This assay has been validated on the Cellomics Arrayscan V<sup>TI</sup> using a 10x objective (0.63X coupler), High Resolution images, XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. The minimally acceptable number of cells used for image analysis in each well was set to 200 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.



**Figure 4.** The FYVE Redistribution assay is very easy and fast to perform.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYORED
074_01	FYVE Redistribution Assay	U2OS	•	•	

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	CRYORED
006_01	Akt1-PH domain Redistribution Assay	CHO	•	•	•
085_01	Akt1 Redistribution Assay	CHO	•	•	
011_02	Akt2 Redistribution Assay	CHO	•	•	•
012_01	Akt3 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	•		
K0100071	Cellomics ERK MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8407901	Cellomics Phospho-c-Jun and Phospho-JNK Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8403701	Cellomics COX-2 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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CelliWoRx	Economical high content reader			

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