

Thermo Scientific Ras Activation 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.

Thermo Scientific Ras Activation Redistribution Assay

GTP-bound Ras activates the Raf/MEK/ERK pathway by recruiting Raf to the plasma membrane. This leads to 14-3-3 protein displacement from the N-terminus of Raf and de-phosphorylation of an otherwise protected negative regulatory site. Ras/Raf interaction is dependent on Ras:GTP binding. This has led to the use of the Ras-binding domain of Raf (RafRBD) fused to glutathione S-transferase (GST) as a capture agent for analyzing the activation state of Ras [1, 2]. The Ras activation Redistribution assay employs RafRBD fused to GFP as a read-out for Ras activity. Active Ras recruits RafRBD-EGFP to the plasma membrane whereas inactive Ras leaves RafRBD-EGFP free in the cytoplasm.

Features

- Designed to assay compounds for their ability to modulate activation of Ras
- 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

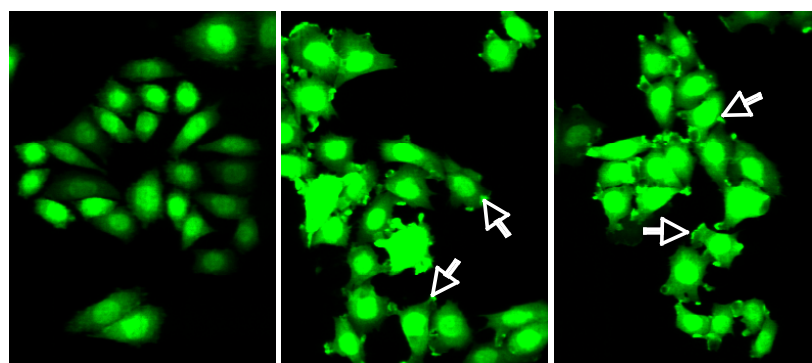


Figure 1. Translocation of RafRBD-EGFP. Cells were untreated (DMSO control, left panel), treated with 100 nM insulin (center panel), or treated with 100 nM IGF-1 (right panel) for 5 min. Arrows indicate the membrane localization detected by the image analysis algorithm.

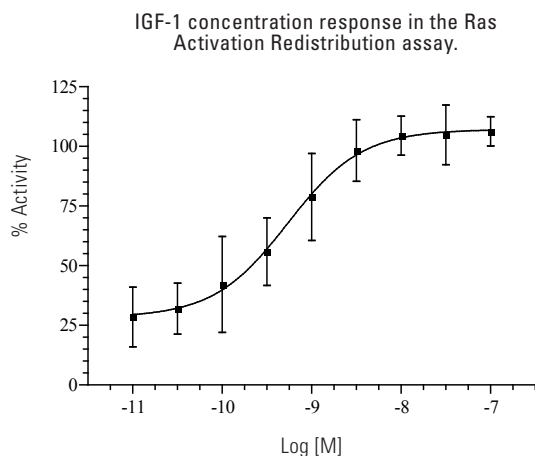


Figure 2. IGF-1 concentration response in the Ras Activation Redistribution assay. Concentration response was measured in 9 point half log dilution series (n = 16). Cells were incubated with IGF-1 for 5 min. Cells were then fixed and the cytoplasm to membrane translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the CytoCellMemTrans.V2 BioApplication. % activity was calculated relative to the positive (100 nM IGF-1) and negative control (0.25% DMSO). The EC₅₀ of IGF-1 is ~0.5 nM.

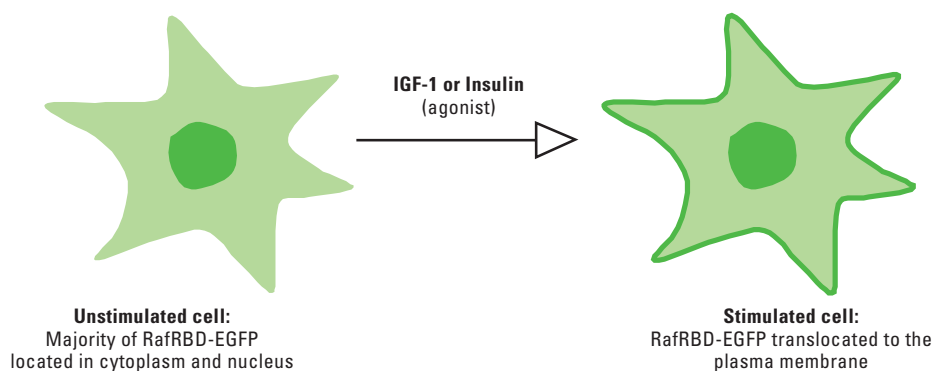


Figure 3. Illustration of the RafRBD-EGFP translocation.

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Assay Details

Recombinant CHOHR cells stably expressing human expressing Raf (1-150) fused to the N-terminus of enhanced green fluorescent protein (EGFP) and Ha-tagged K-Ras. The Ras activation Redistribution assay is sensitive to Ras activation induced by stimulation by IGF-1 or insulin. IGF-1 is used as a reference agonist. Moreover, it is possible to re-format the assay to antagonist format in order to screen for agents that inhibit Ras activation stimulated by insulin or IGF-1. The Ras Activation assay is validated with an average $Z' = 0.26 \pm 0.10$, suitable profiling applications.

Imaging

The translocation of RafRBD-EGFP 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 Ras activation Redistribution assay is the translocation of RafRBD-EGFP 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 300 cells. Other BioApplications that can be used for this assay include ColocalizationV3.

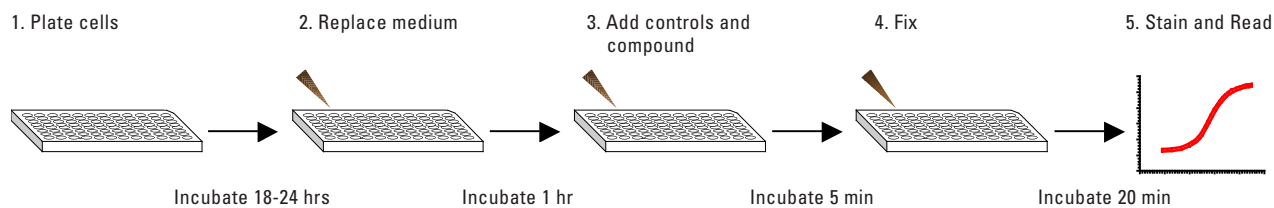


Figure 4. The Ras Activation Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
049_01	Ras Activation Redistribution Assay	CHOHIR	•		

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	CRYOREDI
016_01	STAT3 EGF-stimulated Redistribution Assay	MDA-MB-468	•	•	•
082_01	EGFR Redistribution Assay	U2OS	•		
019_01	ERF, MAPK pathway reporter Redistribution Assay	T24	•	•	•
022_01	ERF, MAPK pathway reporter Redistribution Assay	U2OS	•	•	•
087_01	ERK2 Redistribution Assay	U2OS	•		
K0100071	Cellomics ERK MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404001	Cellomics Phospho-JNK Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100031	Cellomics Phospho-c-Jun 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. Gorman, C. et al. *J. Biol. Chem.* 271: 6713–6719, 1996.
2. Rocks, O. et al. *Curr Opin Cell Biol.* 18:351-7, 2006.

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