

Thermo Scientific MARCKS 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.

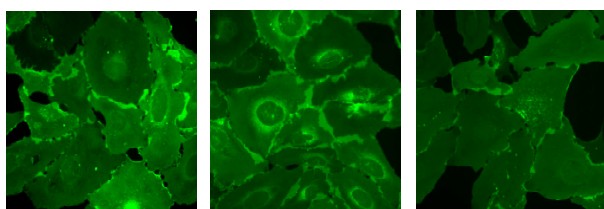


Figure 1. Translocation of MARCKS-EGFP in response to histamine. Cells were treated with 100 μ M histamine for 2 min (middle panel) in the presence (right panel) or absence (DMSO control, left panel) of the antagonist staurosporine. The MARCKS-EGFP translocation from the plasma membrane to perinuclear spots is detected by the image analysis algorithm.

Thermo Scientific MARCKS Redistribution Assay

The protein MARCKS (myristoylated alanine-rich C kinase substrate) is a ubiquitous substrate of PKC. MARCKS is a cytoskeletal protein and has been implicated in regulation of cell spreading, adhesion, phagocytosis, and membrane trafficking [1-3]. MARCKS is localized at the plasma membrane with the N-terminal myristate inserted into the lipid bilayer, but upon activation by PKC it translocates to the cytosol [4].

The MARCKS Redistribution assay has been developed using histamine as reference agonist. Histamine activates endogenous H1 G-coupled receptors, which leads to Ca^{2+} release, PKC activation and ultimately MARCKS translocation from membrane to cytoplasm. Knockdown of endogenous H1 receptors abolishes the response to histamine.

The assay has been developed both in agonist and antagonist format. In the agonist format, histamine is used as reference agonist. In the antagonist assay, the broad PKC inhibitor staurosporine is used as the reference compound and several additional PKC inhibitors have been tested for activity in this assay. GF 109203X, Gö6983, and Ro 31-8220 (all broad isoform specificity) are able to antagonize MARCKS translocation in response of histamine, whereas Gö6976 (conventional PKC specific) is not.

Features

- Designed to assay compounds for their ability to modulate PKC signaling
- 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

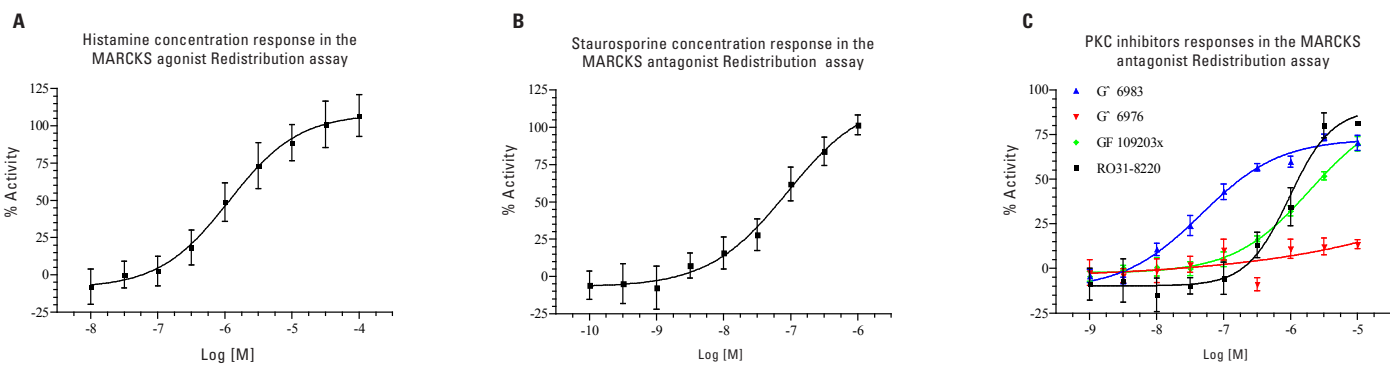


Figure 2. Concentration response curves in the MARCKS assay: A) Histamine concentration response in the MARCKS agonist assay (n=16). The EC_{50} is $\sim 1 \mu\text{M}$. Concentration response was measured in 9 point half log dilution series. Cells were treated with histamine for 2 min. Cells were then fixed and MARCKS translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (100 μM histamine) and negative control (0.25% DMSO). B) Staurosporine concentration response in the MARCKS assay run in antagonist format (n=16). Cells were incubated for 2 min with a half log dilution series of staurosporine in the presence of 100 μM histamine. Cells were then fixed and analyzed on the Cellomics ArrayScan V^{TI} Reader. % activity was calculated relative to the positive (1 μM staurosporine) and negative control (0.25% DMSO). The EC_{50} of staurosporine is $\sim 90 \text{ nM}$. C) Concentration response to a panel of PKC inhibitors in the MARCKS antagonist Redistribution assay (n=4). Cells were incubated for 2 min with a half log dilution series of the PKC inhibitors in the presence of 100 μM histamine. Cells were then fixed and analyzed on the Cellomics ArrayScan V^{TI} Reader. % activity was calculated relative to the positive (1 μM staurosporine) and negative control (0.25% DMSO).

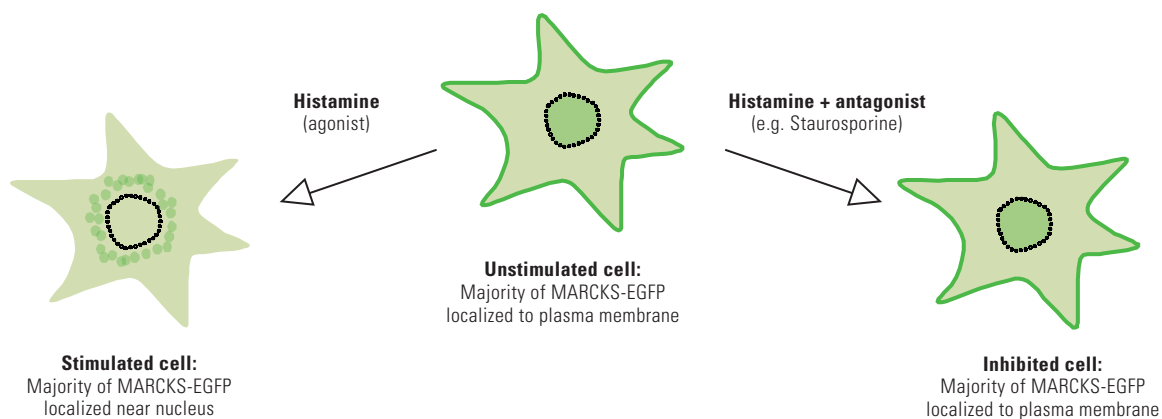


Figure 3. Illustration of the MARCKS translocation event.

Thermo Scientific MARKS Redistribution[®] Assay

Assay Details

Recombinant U2OS cells stably expressing human MARCKS fused to the N-terminus of enhanced green fluorescent protein (EGFP). The MARCKS assay is designed to screen for agonists or antagonists of PKC signaling leading to MARCKS translocation. The MARCKS assay is validated with an average $Z' = 0.40 \pm 0.09$, suitable for screening and profiling applications.

Imaging

The translocation of MARCKS-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 MARCKS Redistribution assay is the formation

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^{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 SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. Two images were taken per well, corresponding to approximately 300 cells/well. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

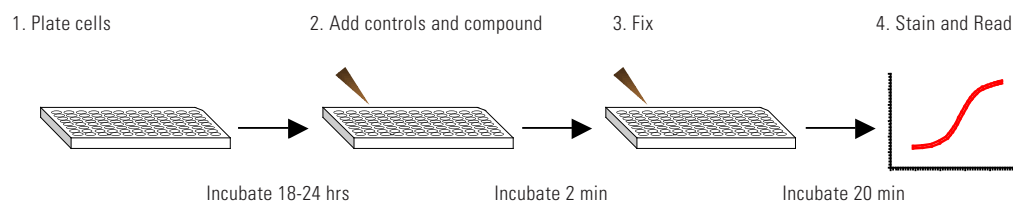
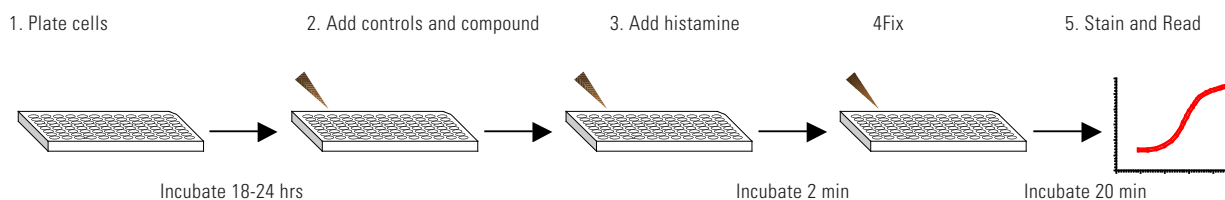
A. Agonist format**B. Antagonist format**

Figure 4. The MARCKS Redistribution assay is very easy and fast to perform in both agonist and antagonist format.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
096_01	MARCKS 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	CRYOREDI
098_01	PKC β Redistribution Assay	U2OS	•		
099_01	PKC ϵ Redistribution Assay	U2OS	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO	•	•	
048_01	NK1:NFATc1 Redistribution Assay	U2OS	•	•	
K0900011	Cellomics PKCalpha Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8402401	Cellomics Cytoskeletal Rearrangement HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0800011	Cellomics Cell Motility 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

- Hartwig JH et al, *Nature*, 1992, Apr 16;356(6370):618-22.
- Blackshear, *JBC*, 1993, 268 (3), Jan 25, 1501-1504
- Aderem, A. *Cell*, 1992. 71, 713–716
- Wang JK et al, *PNAS*, 1989, Apr; 86(7):2253-6

