

Thermo Scientific CRTH2 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.

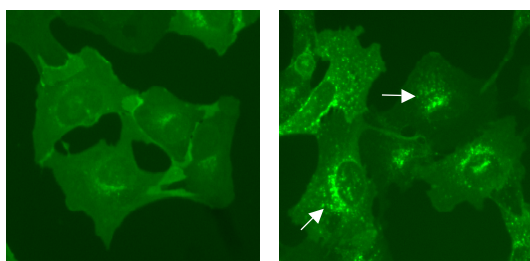


Figure 1. Internalization of CRTH2-EGFP stimulated with Prostaglandin D2. Cells were treated with 300 nM Prostaglandin D2 for 2 hr (right panel) or untreated (DMSO control, left panel). Arrows indicate the CRTH2-EGFP internalization that is detected by the image analysis algorithm.

Thermo Scientific CRTH2 Redistribution Assay

Chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTH2) has attracted interest as a potential therapeutic target in inflammatory and allergic diseases. Prostaglandin D2 has a well-established role in inflammation, and there are two known cellular receptors: DP and CRTH2. They are both members of the superfamily of G protein-coupled receptors (GPCRs). CRTH2 is Gi-coupled, while DP is Gs-coupled. Prostaglandin D2 activation of the CRTH2 receptor causes rapid phosphorylation, desensitization, and endocytosis of CRTH2, with subsequent intracellular processing and recycling to the cell membrane. Evidence for differential regulation of the CRTH2 and D2 receptors

has been published, and small molecule antagonists have been identified. For example, Ramatroban (BAY-u3405) is used in the clinic as a treatment for allergic rhinitis [1-3].

Features

- Designed to assay compounds for their ability to modulate internalization of CRTH2
- 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

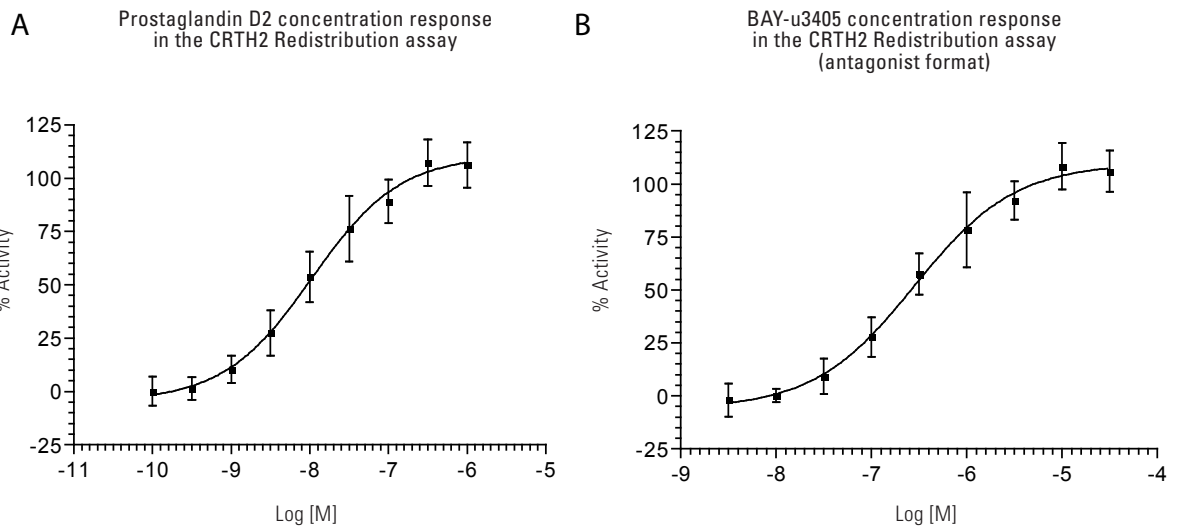


Figure 2. Concentration response curves in the CRTH2 assay: **A)** Prostaglandin D2 concentration response in the CRTH2 agonist assay ($n = 15$). The EC_{50} is ~ 10 nM. Concentration response was measured in 9 point half log dilution series. Cells were treated with prostaglandin D2 for 2 hr. Cells were then fixed and receptor internalization was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (300 nM prostaglandin D2) and negative control (0.25% DMSO). **B)** BAY-u3405 concentration response in the CRTH2 assay run in antagonist format ($n = 8$). Cells were treated with prostaglandin D2 in the presence of a half log dilution series of BAY-u3405 for 2 hr. Cells were then fixed and analyzed on the Cellomics ArrayScan V^{TI} Reader. The EC_{50} of BAY-u3405 is ~ 0.3 μ M.

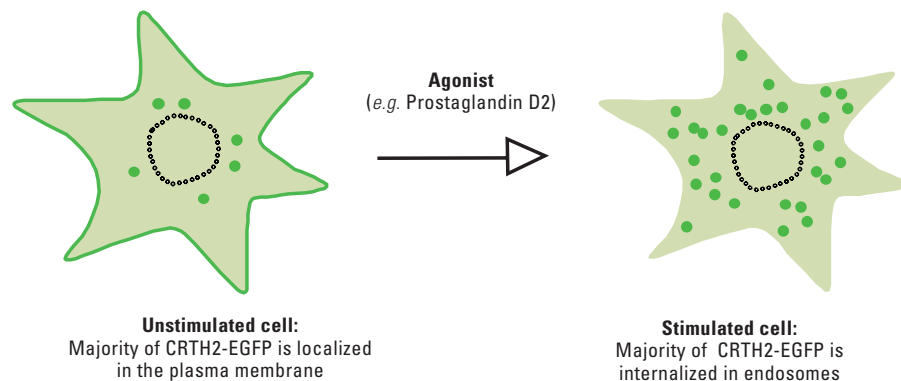


Figure 3. Illustration of the CRTH2 translocation event.

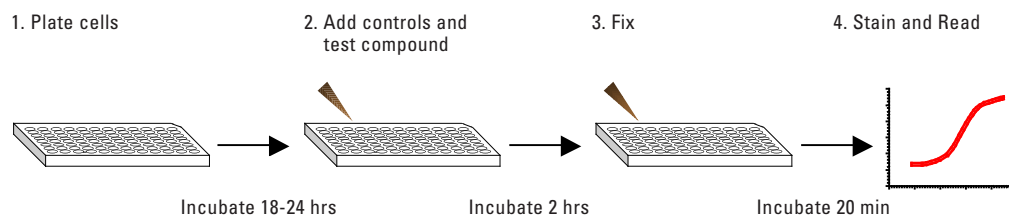


Figure 4. The CRTH2 Redistribution assay is very easy and fast to perform.

Thermo Scientific CRTH2 Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human CRTH2 receptor fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists causing internalization of CRTH2. Prostaglandin D2 is used as reference compound, and ligands/compounds are assayed for their ability to induce CRTH2 internalization by a spot detecting image analysis algorithm. The assay can also be used to detect inhibitors of CRTH2 internalization. The CRTH2 assay is validated with an average $Z' = 0.49 \pm 0.09$, suitable for both screening and profiling applications.

Imaging

The translocation of CRTH2-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 20x objective or higher magnification. The primary output in the CRTH2 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 20x objective (0.63X coupler), 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.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
093_01	CRTH2 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
067_01	CXCR4 Redistribution Assay	U2OS	•	•	
094_01	GRPR Redistribution Assay	U2OS	•	•	
054_01	MCH1 Redistribution Assay	U2OS	•		
039_01	S1P ₁ Redistribution Assay	U2OS	•	•	•
095_01	S1P ₃ Redistribution Assay	U2OS	•		•
086_01	M1 Redistribution Assay	U2OS	•		
075_01	M2 Redistribution Assay	U2OS	•	•	
076_01	M3 Redistribution Assay	U2OS	•	•	
057_01	MC4 Redistribution Assay	U2OS	•		
053_01	FSHR Redistribution Assay	U2OS	•	•	
051_01	CB1 Redistribution Assay	U2OS	•	•	•
061_01	CB2 Redistribution Assay	U2OS	•		•
097_02	GLP1R Redistribution Assay	U2OS	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
088_01	M1:NFATc1 Redistribution Assay	U2OS	•	•	
072_01	M2:PKA Redistribution Assay	CHO-K1	•	•	
073_01	M3:NFATc1 Redistribution Assay	U2OS	•	•	
8404301	Cellomics PKA Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401501	Cellomics Phospho-CREB HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100071	Cellomics ERK MAPK 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. Ulven T & Kostenis E, *Curr Top Med Chem.*, 6:1427-44, 2006.
2. Sugimoto H et al, *J Pharmacol Exp Ther.*, 305:347-52, 2003.
3. Gallant MA, *Eur J Pharmacol.*, 557:115-23, 2007.

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