

Thermo Scientific β2-Adrenergic Receptor 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.

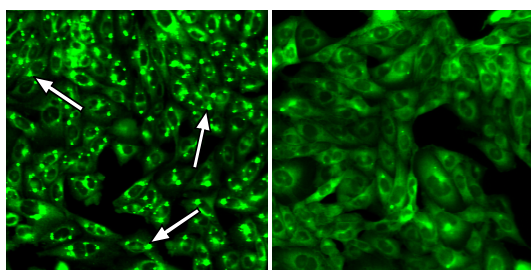


Figure 1. Cytoplasmic foci dispersion of PKAcat-GFP. Cells expressing the β2-adrenergic receptor were treated with 300 nM isoproterenol for 30 min (right panel). Activation of the receptor causes an increase in intracellular cAMP levels, resulting in dispersion of PKAcat-GFP aggregates. Arrows indicate the cytoplasmic foci detected by the image analysis algorithm (DMSO control, left panel).

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β2-adrenergic receptor has its predominant function in lung where receptor agonists are used for relief of bronchoconstriction in treatment of asthma and chronic obstructive pulmonary disease. In this assay the β2-adrenergic receptor has been stably transfected into the GPCR Reporter Assay for Gs-coupled Receptors. Translocation of protein kinase A (PKA), caused by changes in the cytoplasmic cAMP concentration, is used as a reporter for activation of β2-adrenergic receptor. Binding of an agonist to the extracellular parts of β2-adrenergic receptor causes a conformational change in the receptor. This leads to conformational changes in heterotrimeric G proteins at the intracellular face of the receptor, exchange of GDP for GTP on the alpha subunit

(Gαs) and subsequent release of Gαs from the beta-gamma subunit. GTP-bound Gαs diffuses into the cytoplasm where it activates adenylate cyclase, which then catalyzes the formation of cAMP from ATP. In turn, cAMP activates PKA.

Features

- Designed to assay compounds for their ability to activate a cAMP response through β2-Adrenergic Receptor
- 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

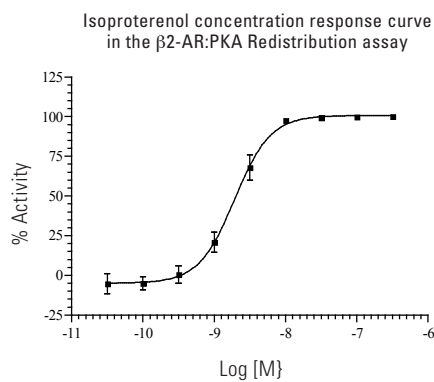


Figure 2. Isoproterenol concentration response in the β2-AR:PKA assay. Concentration response was measured in 9 point half log dilution series (n = 8). The EC₅₀ of isoproterenol is ~2 nM. Cells were treated with isoproterenol for 30 min. Cells were then fixed and cytoplasmic spot formation was measured using the Cellomics ArrayScan V^{Hi} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (300 nM isoproterenol) and negative control (0.25% DMSO).

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

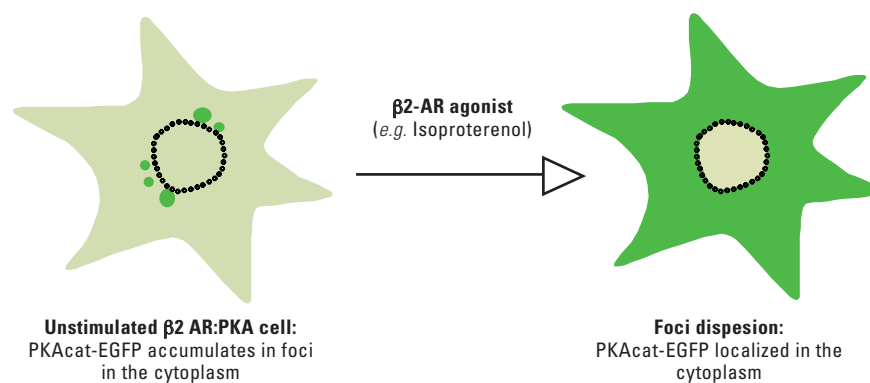


Figure 3. Illustration of the PKAcat-EGFP translocation event.

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Assay Details

Recombinant CHO-K1 cells stably expressing the β2-Adrenergic receptor (β2-AR) and the catalytic domain of human Protein Kinase A (PKAcat) fused to the N-terminus of enhanced green fluorescent protein (EGFP). In unstimulated cells, PKAcat-EGFP is found in highly fluorescent aggregates in the cytoplasm. Activation of PKA by cAMP leads to release of the PKAcat-EGFP fusion protein from the aggregates, resulting in the disappearance of fluorescent spots in the cytoplasm [1-3]. The β2-AR:PKA assay is designed to screen for compounds causing dispersion of PKAcat-EGFP aggregates. Such compounds are considered to be agonists for β2-adrenergic receptor activation. Isoproterenol is used as reference compound in the assay. The β2-Adrenergic Receptor Activation assay is validated with an average $Z' = 0.77 \pm 0.05$, suitable for both screening and profiling applications.

Imaging

The translocation of PKAcat-GFP 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 β2-AR:PKA Redistribution assay is the dispersion 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^{PI}

This assay has been validated on the Cellomics Arrayscan V^{PI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotTotalIntenPerObject. 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 CompartmentalAnalysisV2 and ColocalizationV3.

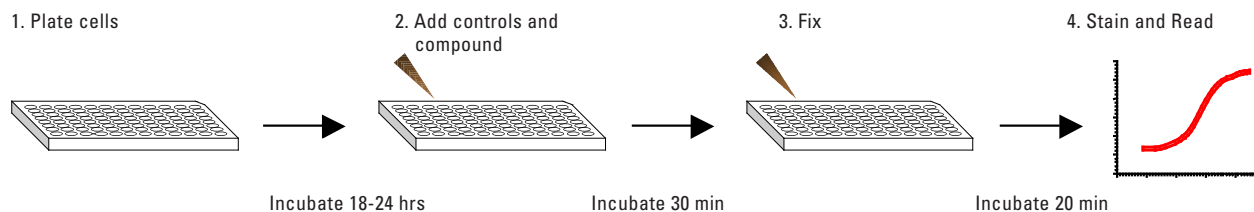


Figure 4. The β 2-Adrenergic Receptor Activation Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
046_01	β 2-Adrenergic Receptor Activation 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	CRYOREDI
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
047_01	GlucagonR:PKA Redistribution Assay	CHO-K1	•	•	
048_01	NK1:NFATc1 Redistribution Assay	U2OS	•	•	
067_01	S1P1: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	•	•	
078_01	AT1:NFATc1 Redistribution Assay	U2OS	•	•	
079_01	MCH1:NFATc1 Redistribution Assay	U2OS	•	•	
081_01	MOR1:PKA Redistribution Assay	CHO-K1	•	•	
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			
K0100101	Cellomics ATF-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 ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. Feliciello, A. et al., *J. Mol. Biol.*; 308, 99-114, 2001.
2. Almholt, K. et al., *Cell Signal.*; 16, 907-20, 2004.
3. Zaccolo, M. et al., *Nat. Cell Biol.*; 2, 25-29, 2000.

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