

Thermo Scientific M2:PKA 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.

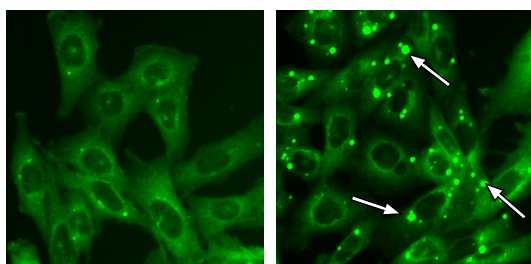


Figure 1. Formation of cytoplasmic foci of PKAcat-GFP. Cells expressing the M2 receptor were treated with 40 μ M forskolin for 5 min in the presence (right panel) or absence (left panel) of 10 μ M carbachol. Activation of the receptor inhibits the forskolin-induced dispersion of PKAcat-GFP aggregates. Arrows indicated the cytoplasmic foci detected by the image analysis algorithm.

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Muscarinic acetylcholine receptor 2 (M2) belongs to a family of G protein-coupled receptors (GPCRs) which consists of five members. The odd-numbered members (M1, M3, M5) couple to $G\alpha_q$ whereas the even-numbered members (M2, M4) couple to $G\alpha_i$. Muscarinic acetylcholine receptors mediate the effect of acetylcholine in synapses, and they are involved several indications related to the central nervous system [1].

In this assay, the M2 receptor is stably transfected into the PKAcat GPCR Reporter Assay for Gs/Gi-coupled Receptors (Product ID 045_02). This assay system measures translocation of protein kinase A (PKA) in response to changes in the cytoplasmic cAMP concentration. Binding of an agonist to the extracellular parts of the M2 receptor causes activation of the Gi complex at the intracellular face of the receptor. This leads to inhibition of adenylyl cyclase, which catalyzes the formation of cAMP from ATP. In cells stimulated with forskolin that induces high levels of cAMP, PKAcat-

GFP is found in a diffuse localization in the cytoplasm. M2 activation leads to inhibition of adenylyl cyclase and thereby less cAMP. This, in turn, leads to aggregation of PKAcat-GFP in cytoplasmic foci [2-4].

Features

- Designed to assay compounds for their ability to inhibit a cAMP response through the M2 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

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

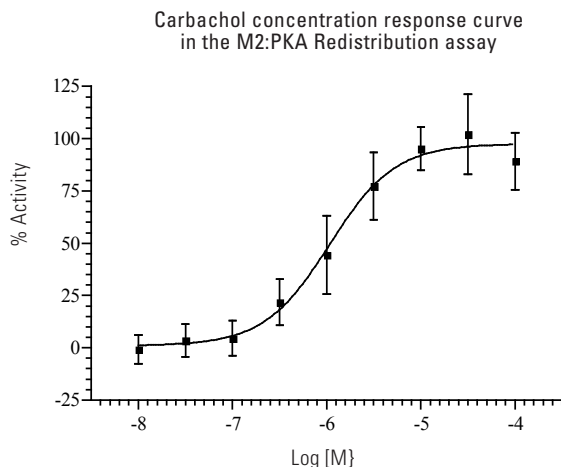


Figure 2. Carbachol concentration response in the M2:PKA assay. Concentration response was measured in 9 point half log dilution series ($n = 24$). The EC_{50} of carbachol is $\sim 1 \mu\text{M}$. Cells were treated with carbachol in the presence of forskolin for 5 min. Cells were then fixed and cytoplasmic spot formation was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive ($10 \mu\text{M}$ carbachol) and negative control (0.25% DMSO).

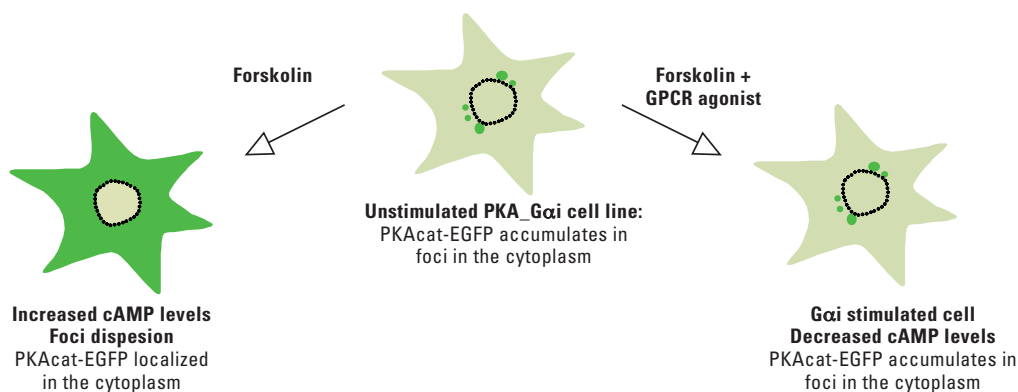


Figure 3. Illustration of the PKAcat translocation event.

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Assay Details

Recombinant CHO-K1 cells stably expressing the muscarinic acetylcholine receptor 2 (M2) and the catalytic domain of human Protein Kinase A (PKAcat) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists causing formation of PKAcat-GFP aggregates. Carbachol is used as reference compound. The M2:PKA assay is validated with an average $Z' = 0.61 \pm 0.10$, suitable for both screening and profiling applications.

Imaging

The translocation of PKAcat-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 M2:PKA 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 SpotDetectorV2 BioApplication. The output parameter used was SpotCountPerObject. The minimally acceptable number of cells used for image analysis in each well was set to 400 cells. Other BioApplications that can be used for this assay include SpotDetectorV3, CompartmentalAnalysisV2, and ColocalizationV3.

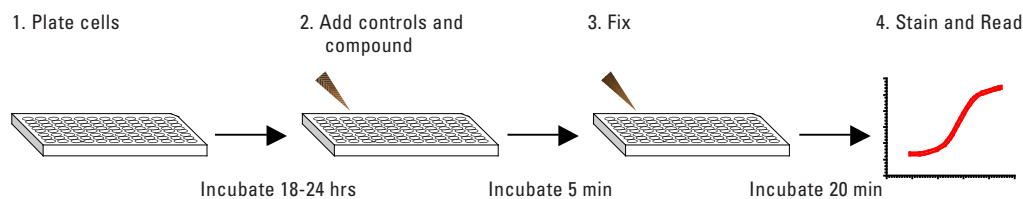


Figure 4. The M2:PKA Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
072_01	M2:PKA Redistribution Assay	CHO-K1	•	•	

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	•	•	
046_01	β 2-AR: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	•	•	
081_01	MOR1: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	•	•	
047_01	GlucagonR: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			
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

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3. Almholt, K. et al., *Cell Signal.*; 16, 907-20, 2004.
4. Zaccolo, M. et al., *Nat. Cell Biol.*; 2, 25-29, 2000.

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