

Thermo Scientific PKA Gs/Gi-coupled GPCR Reporter 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.

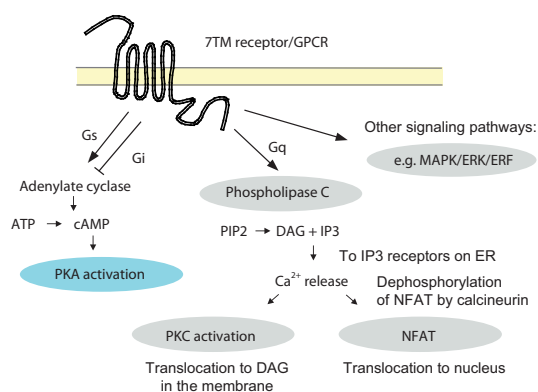


Figure 1: Schematic overview of GPCR signaling. Gs and Gi coupled GPCRs regulate the formation of cAMP through activation (Gs) or inhibition (Gi) of adenylate cyclase. High levels of cAMP lead to activation of PKA and translocation of the PKA catalytic domain from cytoplasmic foci to a uniform cytoplasmic localization.

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The GPCR Reporter Assay for Gs-coupled Receptors uses protein kinase A (PKA) translocation, caused by changes in the cytoplasmic cAMP concentration, as a reporter for activation of Gs-coupled GPCRs. Binding of an agonist to the extracellular parts of a Gs-coupled GPCR causes a conformational change in the receptor. This leads to conformational changes in heterotrimeric Gs proteins at the intracellular face of the receptor, exchange of GDP for GTP on the alpha subunit ($G\alpha_s$), and subsequent release of $G\alpha_s$ from the beta-gamma subunit. GTP-bound $G\alpha_s$ activates adenylate cyclase which then catalyzes the formation of cAMP from ATP. In turn, cAMP activates PKA (Figure 1). Gi-coupled receptors activate $G\alpha_i$ in a similar way; however $G\alpha_i$ acts as an inhibitor of adenylate cyclase, decreasing cAMP levels.

PKA is an ubiquitous serine/threonine protein kinase, and a major mediator of intracellular cAMP signals in eukaryotes. The PKA holoenzyme is an R2C2 tetramer consisting of a regulatory (R) dimer and two catalytic (C) subunits. The catalytic subunits in the assembled tetramer are generally believed to be catalytically inactive. Dissociated catalytic subunits are freely mobile and phosphorylate cytoplasmic substrates [1].

Features

- Designed to assay compounds for their ability to modulate activation of Gs-G11-coupled GPCRs via PKA translocation
- 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

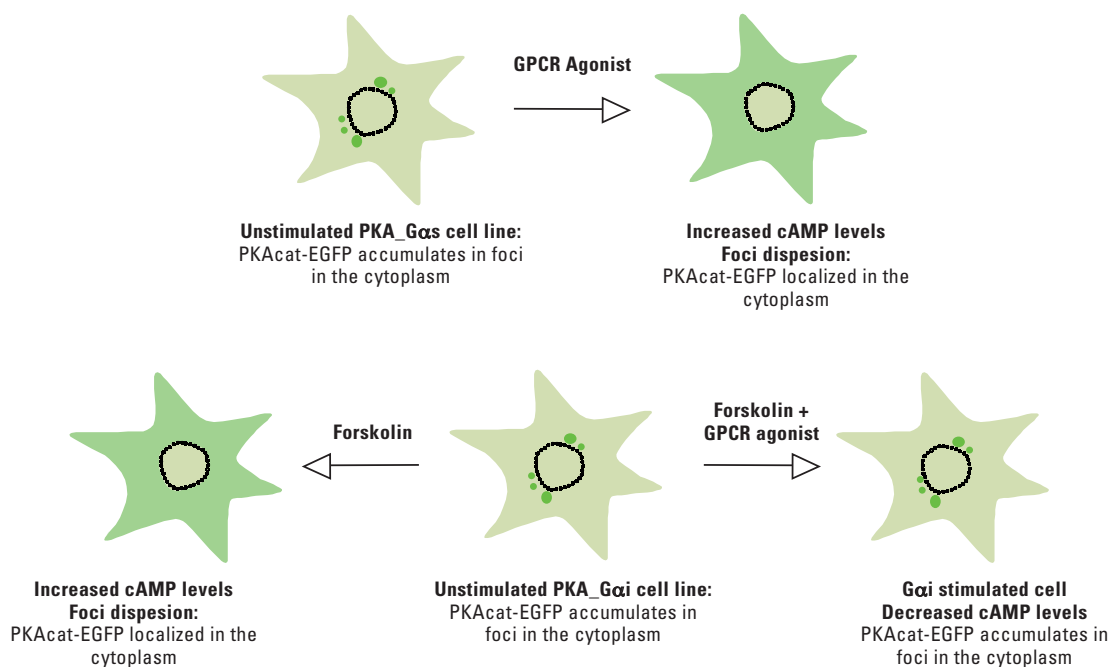


Figure 2. Illustration of how the reporter cell line (top) and PKA Gi GPCR reporter cell line (below) are used and the PKAcacat-GFP translocation event in response to activation of user defined GPCR.

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Assay Details

Recombinant CHO-K1 cells stably expressing the catalytic domain of human Protein Kinase A (PKAcacat) fused to the N-terminus of enhanced green fluorescent protein (EGFP). In the assay cell line, the catalytic domain of PKA is fused to EGFP (PKAcacat-EGFP). In unstimulated cells, PKAcacat-EGFP is found in highly fluorescent aggregates in the cytoplasm. Activation of PKA by cAMP leads to release of the PKAcacat-EGFP fusion protein from the aggregates, resulting in the disappearance of fluorescent spots in the cytoplasm [2,3].

The PKA Gs/Gi-coupled GPCR Redistribution cell line is developed to be used as a parental cell line to build specific GPCR assays after transfection of GPCR of interest. Figure 2 illustrates the translocation of PKAcacat-EGFP upon agonist stimulation of a Gs or Gi-coupled GPCR. Test compounds causing dispersion of PKA aggregates are considered agonists for the GPCR that has been transiently or stably transfected into the assay cell line. Optimization of assay parameters is necessary for creating a robust cell line with Gs coupled receptors, additionally selection of single cell clone is required for Gi coupled receptors.

Imaging

The dispersion of PKA aggregates 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 is the formation/dispersion of spots in the cytoplasm. The data analysis should therefore report an output that corresponds to area or intensity of spots in the cytoplasm.

Imaging on Thermo Scientific Cellomics ArrayScan V[®]

This assay has been tested on the Cellomics Arrayscan V[®] 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 250 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

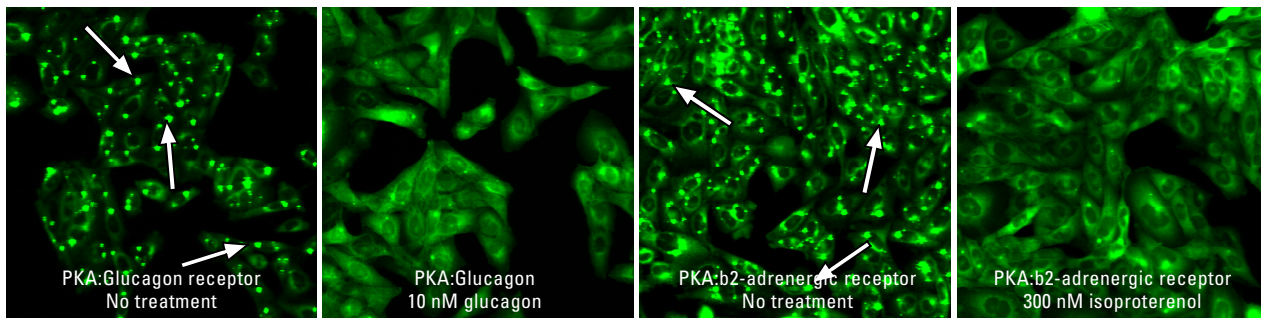


Figure 3. Images of PKAcet-GFP expressing cells stably transfected with glucagon receptor or β 2-adrenergic receptor. Cells have been treated in the absence (DMSO) or presence of receptor agonist. Arrows indicate PKAcet-GFP foci in the cytoplasm of non-treated cells detected by a spot-detecting image analysis algorithm

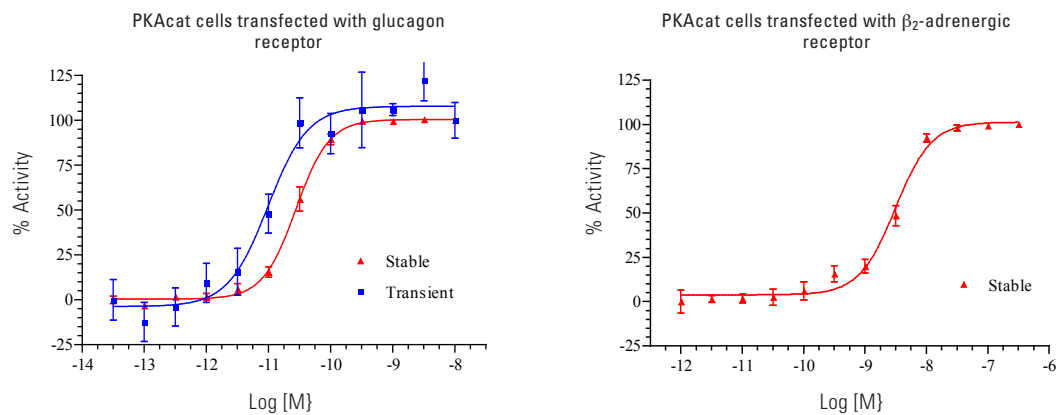


Figure 4. Concentration response curves for glucagon and isoproterenol in the PKA Gs/Gi GPCR reporter cell line transfected with glucagon receptor or β 2-adrenergic receptor. A) Glucagon receptor was transfected transiently or stably into the PKA Gs/Gi GPCR reporter cell line. Transfected cells were treated with glucagon in concentration response for 30 min. The EC_{50} value of glucagon is approximately 10 pM for transiently transfected cells and 27 pM for stably transfected cells. % activity was calculated relative to the positive (10 nM glucagon) and negative control (0.25% DMSO) ($n = 4$). B) β 2-adrenergic receptor was transfected stably into the PKA Gs/Gi GPCR reporter cell line. Transfected cells were treated with isoproterenol in concentration response for 30 min. The EC_{50} value of isoproterenol is approximately 3 nM. % activity was calculated relative to the positive (300 nM isoproterenol) and negative control (0.25% DMSO) ($n=4$).

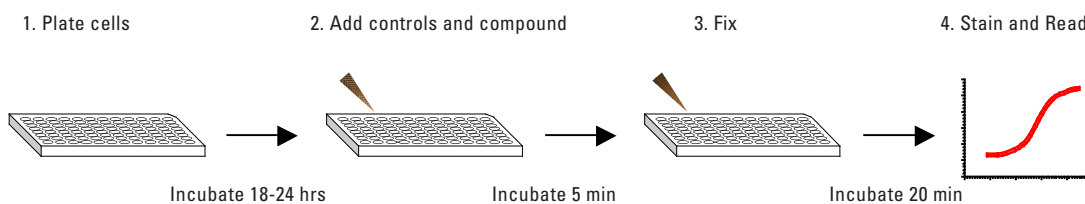


Figure 5. The Gs/Gi-coupled GPCR Reporter Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
045_02	Gs/Gi-coupled GPCRs – 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	•	•	
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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