

Thermo Scientific Glucagon 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.

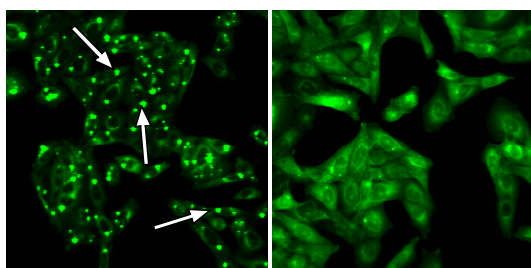


Figure 1. Cytoplasmic foci dispersion of PKAcat-GFP. Cells expressing the glucagon receptor were treated with 10 nM glucagon for 30 min (right panel). Activation of the receptor causes an increase in intracellular cAMP levels, resulting in dispersion of PKAcat-GFP aggregates. Arrows indicated the cytoplasmic foci detected by the image analysis algorithm (DMSO control, left panel).

Thermo Scientific Glucagon Receptor Activation Redistribution Assay

Glucagon and glucagon receptor are involved in energy homeostasis through stimulation of gluconeogenic pathways in the liver, causing blood glucose levels to increase. In this assay the glucagon 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 glucagon receptor. Binding of an agonist to the extracellular parts of glucagon receptor 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 α s) and subsequent release of G α s from the beta-gamma subunit.

GTP-bound G α s 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 Glucagon 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

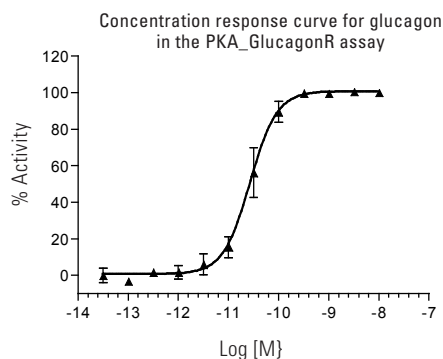


Figure 2. Glucagon concentration response in the GlucagonR:PKA assay. Concentration response was measured in 9 point half log dilution series (n = 8). The EC₅₀ of glucagon is ~30 pM. Cells were treated with glucagon for 30 min. Cells were then fixed and cytoplasmic spot formation was measured using the IN Cell Analyzer 3000 (GE Healthcare). % activity was calculated relative to the positive (10 nM glucagon) 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

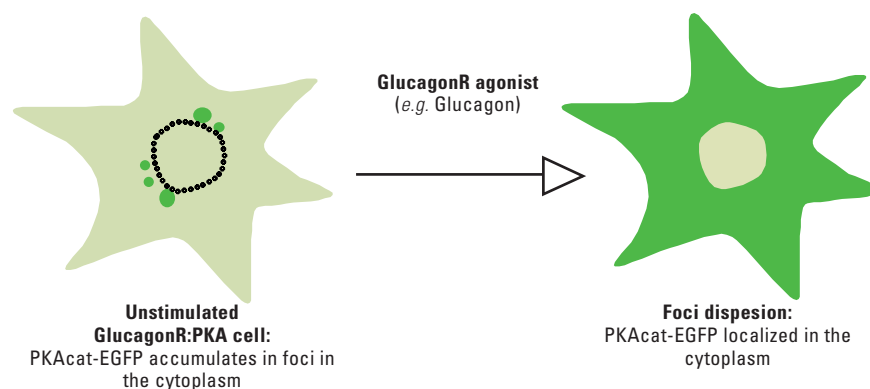


Figure 3. Illustration of the PKAcat-EGFP translocation event.

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Assay Details

Recombinant CHO-K1 cells stably expressing the glucagon receptor 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 GlucagonR:PKA assay is designed to screen for agonists causing dispersion of PKAcat-GFP aggregates. Such compounds are considered to be agonists for glucagon receptor activation. Glucagon is used as the reference ligand in the assay. The Glucagon Receptor Activation assay is validated with an average $Z' = 0.78 \pm 0.13$, suitable for both screening and profiling applications.

primary output in the GlucagonR: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^{TI}

This assay is recommended to be run on the Cellomics ArrayScan V^{TI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication, using the output parameter SpotTotalIntenPerObject. The minimally acceptable number of cells used for image analysis in each well should be set to 100 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

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

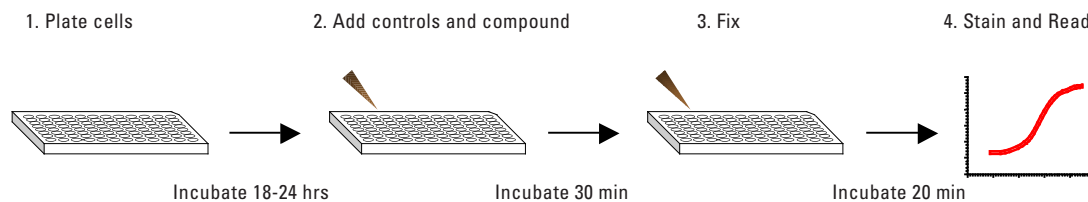


Figure 4. The Glucagon Receptor Activation Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
047_01	Glucagon Receptor Activation 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	•	•	
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			
8405201	Cellomics Phospho-S6 Detection 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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