

Thermo Scientific 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.

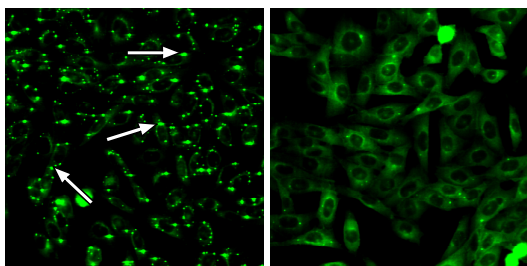


Figure 1. Translocation of PKAcat-GFP. Cells were treated without (DMSO control, left panel) or with 50 μ M forskolin (right panel) for 5 min. Arrows indicate the aggregates of PKAcat-EGFP in the cytoplasm of untreated cells detected by the image analysis algorithm.

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The cAMP-dependent protein kinase A (PKA) is a ubiquitous serine/threonine protein kinase. PKA is recognised as 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. Four isoforms of the regulatory subunit (RI α , RI β , RII α , RII β) and three isoforms of the catalytic subunit (C α , C β , and C γ) have been described. The catalytic subunits in the assembled tetramer are generally believed to be catalytically inactive. Gs-protein activation leads to activation of adenylate cyclases, which generate cAMP. Binding of two molecules of cAMP to each R subunit causes the release and activation of the C subunits. Dissociated catalytic subunits phosphorylate cytoplasmic substrates.

To date, a large number of cytoplasmic and a few nuclear PKA substrates have been reported. The amino-termini of the catalytic subunits are myristoylated, a post-translational modification usually associated with membrane attachment. However, catalytic subunits are freely mobile within cells when detached from the regulatory subunits [1].

Features

- Designed to assay compounds for their ability to modulate activation of PKA
- 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

Forskolin concentration response curve in the PKA Redistribution assay

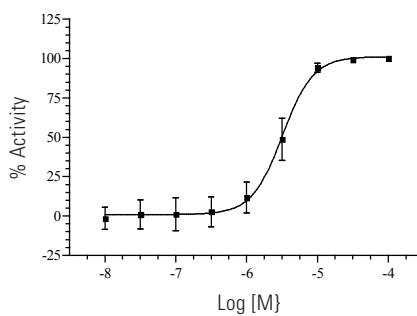


Figure 2. Concentration response curve of forskolin in the PKA assay (n = 16). The EC₅₀ is approximately 3 μ M. Concentration response was measured in 9 point half log dilution series. Cells were treated with compound for 5 min. Cells were then fixed and spot dispersion was measured using imaging on the Cellomics ArrayScan V[™] Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (50 μ M forskolin) 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

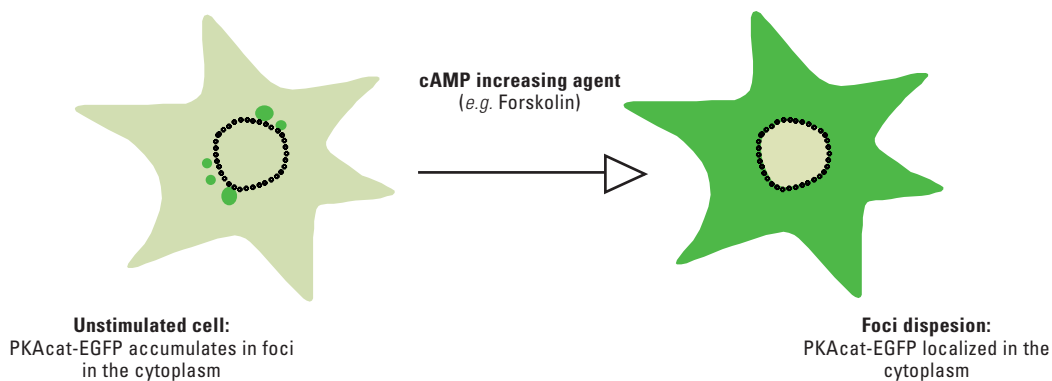


Figure 3. Illustration of the PKA translocation event.

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Assay Details

Recombinant CHO-K1 cells stably expressing the catalytic domain of human Protein Kinase A (PKAcac) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The PKA Redistribution assay is designed to assay for the concentration of cAMP in the cytoplasm by monitoring translocation of the catalytic domain of PKA fused to GFP (PKAcac-EGFP) from cytoplasmic aggregates to a uniform cytoplasmic localization [2,3]. In unstimulated cells, the PKAcac-GFP is found in highly fluorescent aggregates in the cytoplasm. Upon stimulation with forskolin, an activator of adenylate cyclase, cAMP is generated, leading to cAMP binding and activation of PKAcac-EGFP which results in dispersion of PKAcac-EGFP from the bright aggregates. Forskolin is used as reference compound in the assay [4]. Test compounds causing foci dispersion of PKA are considered activators of adenylate cyclase leading to generation of cAMP. The PKA assay is validated with an average $Z' = 0.67 \pm 0.08$, suitable for both screening and profiling applications.

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 validated 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.

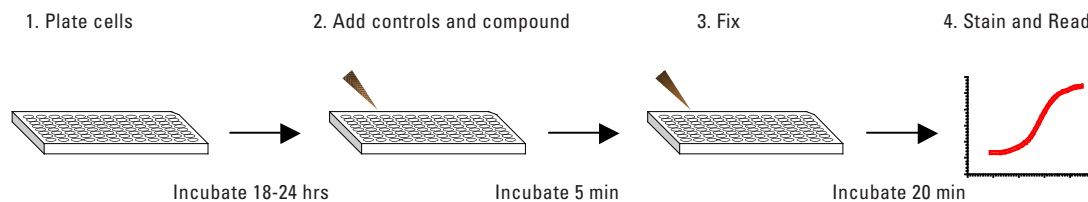


Figure 4. The PKA Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
045_01	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	•	•	
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.
4. Seamon KB & Daly JW., *Adv Cyclic Nucleotide Protein Phosphorylation Res.* 20, 1-150, 1986.

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