

Thermo Scientific PKC β 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.

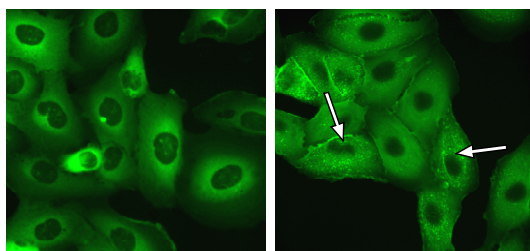


Figure 1. Translocation of PKC β -EGFP. U2OS cells expressing the NK1 receptor and PKC β -EGFP (DMSO control, left panel) were treated with 3 nM Substance P (right panel). Activation of the NK1 receptor leads to release of Ca²⁺ and DAG which in turn induces translocation of PKC β -EGFP from the cytoplasm to spots in the cytoplasm and the plasma membrane. Arrows indicate the translocation detected by the image analysis algorithm.

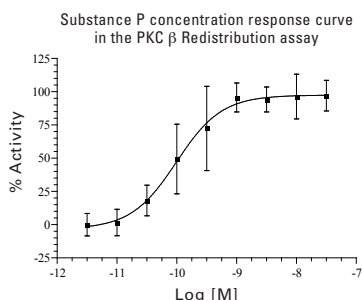


Figure 2. Substance P concentration response in the PKC β assay. Concentration response was measured in 9 point half log dilution series (n=16). The EC₅₀ of Substance P is approximately 100 pM. Cells were treated with Substance P and then fixed immediately. Translocation was measured using the Cellomics ArrayScan V[®] Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (3 nM Substance P) 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

Thermo Scientific PKC β Redistribution Assay

Protein Kinase C is an expanding family of at least 10 enzymes that can be divided into three classes. The conventional kinases (α , β I, β II, γ), the novel (δ , ϵ , η , θ , μ), and the atypical (ζ , ι , λ). The kinases are localized to the cytoplasm, but upon activation they translocate to an organelle or the plasma membrane. The conventional PKC isoforms are activated by Ca²⁺ and 1,2-diacylglycerol (DAG), the novel isoforms require DAG for activation, and activation of the atypical isoforms is less described. A common mechanism of Ca²⁺ and DAG release is through activation of G_q-coupled GPCRs. A large number of PKC substrates are then phosphorylated and thereby regulated by PKC. PKC β has been reported to be involved in many different cellular functions, for

example B cell activation, apoptosis, and diabetic dysfunctions [1-3].

Features

- Designed to assay compounds for their ability to modulate activation of PKC β
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope application

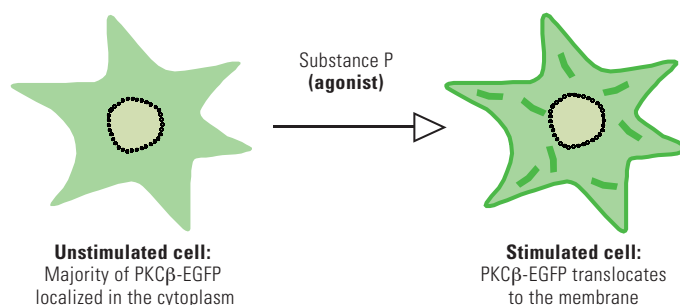


Figure 3. Illustration of the PKC β translocation event.

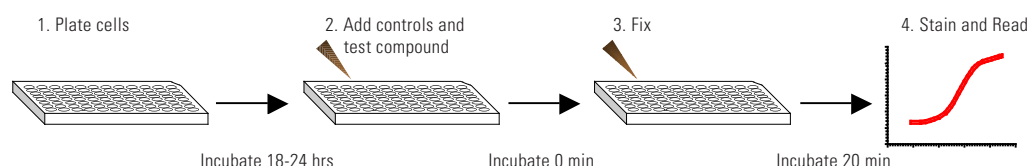


Figure 4. The PKC β Redistribution assay is very easy and fast to perform.

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Assay Details

Recombinant U2OS cells stably expressing human PKC β fused to the N-terminus of enhanced green fluorescent protein (EGFP). The PKC β Redistribution assay is developed using Substance P as agonist. Substance P activates coexpressed NK1 receptors, which activate Gq, PLC and subsequently PKC β . Compounds can be tested for agonistic effect on the PKC β pathway. The assay can be reformatted to an antagonist assay format, for testing PKC β pathway inhibitors. The PKC β assay is validated with an average $Z' = 0.28 \pm 0.05$, suitable for profiling applications.

Imaging

The translocation of PKC β 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

20x objective or higher magnification.

The primary output in the PKC β Redistribution assay is the formation of spots in the cytoplasm and the plasma membrane. The data analysis should therefore report an output that corresponds to number, area, or intensity of these spots.

Imaging on Thermo Scientific Cellomics ArrayScan V^{ti}

This assay has been validated on the Cellomics ArrayScan V^{ti} using a 20x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. The minimally acceptable number of cells used for image analysis in each well was set to 150 cells.

Other BioApplications that can be used for this assay include Cytoplasm to Cell Membrane V2, CompartmentalAnalysisV2, and ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYORED1
098_01	PKC β Redistribution Assay	U2OS	•		

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	CRYORED1
099_01	PKC ϵ Redistribution Assay	U2OS	•	•	
096_01	MARCKS Redistribution Assay	U2OS	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO	•	•	
048_01	NK1:NFATc1 Redistribution Assay	U2OS	•	•	
K0900011	Cellomics PKC α 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

- Leitges et al, *Science* 273: 788-791, 1996
- Su et al, *Nature Immun.* 3: 780-786, 2002.
- Ishii et al, *Science*. 1996 May 3; 272 (5262):728-31

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