Product Specifications

Thermo Scientific PDE4A4 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.

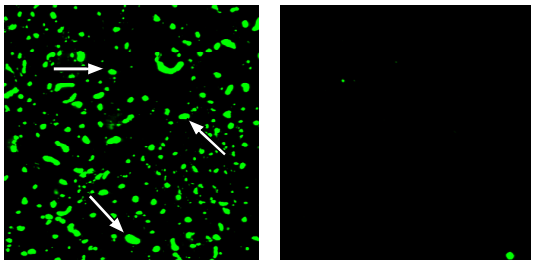

Figure 1. Translocation of PDE4A4-EGFP. Cells that were preincubated with RS25344 were treated with (right panel) or without (left panel) 10 µM RP73401 for 2 hr. Arrows indicate the cytoplasmic aggregates of PDE4A4-EGFP that are detected by the image analysis algorithm.

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 PDE4A4 Redistribution Assay

cAMP and cGMP are ubiquitous second messengers with important regulatory roles in a wide variety of signal transduction pathways and in various tissues. The intracellular levels of cAMP and cGMP are tightly controlled both by their rate of synthesis by adenylyl and guanylyl cyclases, respectively, in response to extracellular signals, and by their rate of hydrolysis by cyclic nucleotide phosphodiesterases (PDEs) [1]. PDEs form a superfamily of enzymes that catalyze the hydrolysis of 3′,5′-cyclic nucleotides to the corresponding nucleotide 5′-monophosphates. Eleven distinct PDE families have been identified containing more than 50 different PDE enzyme variants.

PDEs contain a conserved catalytic domain, but exhibit different substrate preferences, distinct tissue, cell, and subcellular expression patterns and therefore participate in distinct physiological and pathophysiological processes such as CNS function, cardiovascular function, inflammatory cells/immune system, cell adhesion as well as metabolic processes. More than 16 different PDE4, cAMP-specific isoforms have been identified and four separate genes (A, B, C, and D) encode these various isoforms. Selective inhibitors against the PDE4 isoforms are of interest as anti-asthma therapeutics, anti-depressants, and in the treatment of inflammation and chronic obstructive pulmonary disease.

The PDE4A4 Redistribution assay is based on distinct translocation behaviour of the human cAMP phosphodiesterase PDE4A4 [2]. Treatment with the PDE4A4 Redistribution agonist RS25344 leads to a localization of PDE4A4 in compact foci [3]. Focus formation of PDE4A4 may be of use in probing for conformational changes in this isoform and for sub-categorising PDE4 selective inhibitors. Furthermore, the PDE4A4 Redistribution assay functions as a counter-screening assay for our GRIP technology based assays (see related products).

Features

- Designed to assay compounds for their ability to modulate translocation of PDE4A4 to cytoplasmic foci
- 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

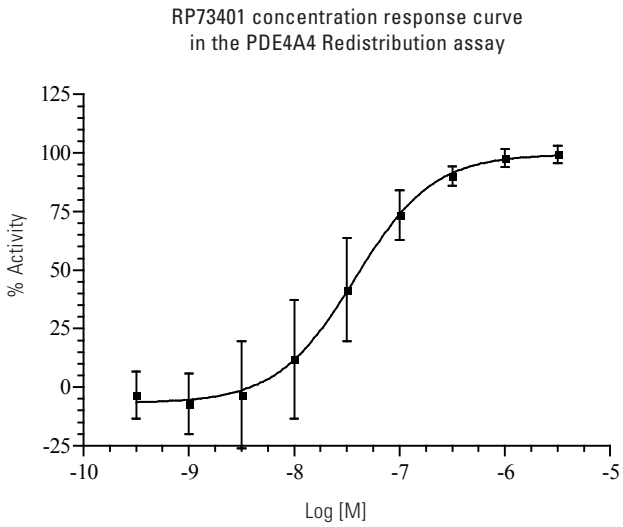

Figure 2. Concentration response curve of RP73401 in the PDE4A4 assay (n = 24). The EC_{50} of RP73401 is approximately 40 nM. Concentration response was measured in 9 point half log dilution series. Cells were treated with compound for 2 hr. Cells were then fixed and spot dispersion was measured using imaging on the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (10 µM RP73401) and negative control (0.25% DMSO).

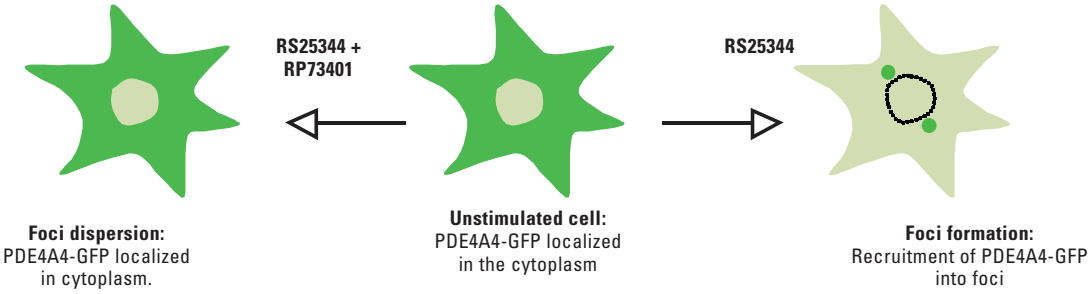

Figure 3. Illustration of the PDE4A4 translocation event.

Thermo Scientific PDE4A4 Redistribution® Assay

Assay Details

Recombinant CHOhIR cells stably expressing human phosphodiesterase 4A4B (PDE4A4) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to monitor the translocation of PDE4A4 to cytoplasmic foci. The PDE4A4 Redistribution antagonist, RP73401, inhibits RS25344-induced foci formation and is used as reference compound in this assay to disperse PDE4A4 foci [4]. The PDE4A4 assay is validated with an average Z' = 0.63 ± 0.09, suitable for both screening and profiling applications.

Imaging

The translocation of PDE4A4-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 PDE4A4 Redistribution assay is the dissociation 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 SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. The minimally acceptable number of cells used for image analysis in each well was set to 200 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

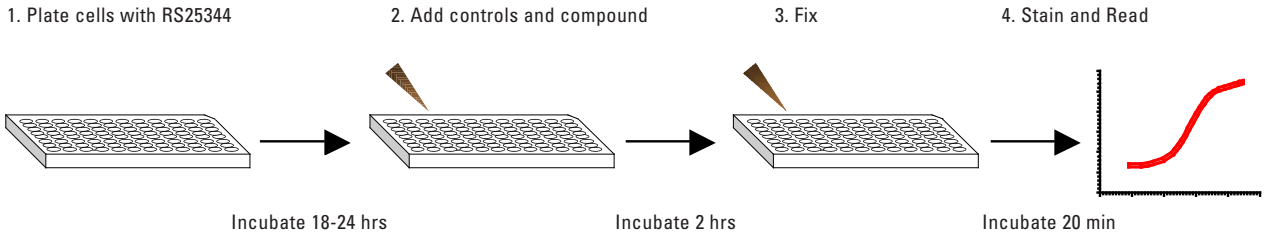

Figure 4. The PDE4A4 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
029_01	PDE4A4 Redistribution Assay	CHO	•	•	

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
045_01	Protein Kinase A Redistribution Assay	CHO-K1	•	•	
020_01	p53-Hdm2 GRIP Redistribution Assay	CHOhIR	•	•	
8401501	Cellomics Phospho-CREB HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404301	Cellomics PKA Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8403701	Cellomics COX-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. Houslay MD & Adams DR, *Biochem J.* 370, 1-18, 2003.
2. Terry B et al., *Cell Signal.* 15, 955-971, 2003.
3. Alvarez R et al., *Mol. Pharmacol.* 48, 616-622, 1995.
4. Ashton MJ et al., J. *Med. Chem.* 37, 1696-703, 1994.

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