Product Specifications

Thermo Scientific S1P$_3$ 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.

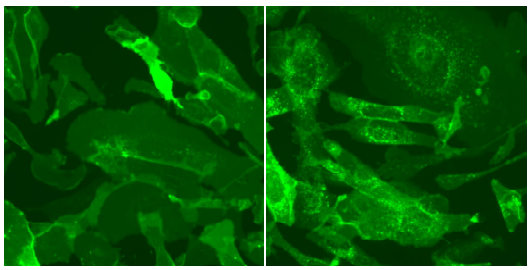

Figure 1. Internalization of S1P$_3$-EGFP stimulated with S1P. Cells were treated with 10 µM S1P for 1 hr (right panel) or untreated (DMSO control, left panel). The cytoplasmic spots are detected by the image analysis algorithm.

Thermo Scientific S1P$_3$ Redistribution Assay

Sphingosine-1-phosphate (S1P) is a pleiotropic platelet-derived lysophospholipid involved in the regulation of cell growth and differentiation, thereby important for angiogenesis, embryogenesis, and atherosclerosis [1,2]. S1P acts on five subtypes of G-protein-coupled receptors (S1P$_{1\text{-}5}$). S1P$_3$, formerly named endothelial differentiation gene-3 (EDG3), is expressed in many cell types, including endothelial and vascular smooth muscle cells. Binding of S1P to the S1P$_3$ receptor activates G_i, G_q,and $G_{12/13}$ subsets of the G-protein families [3]. It has recently been suggested that S1P-induced inflammatory responses are mediated by both S1P$_1$ and S1P$_3$ suggesting that these receptors contribute to the regulation of angiogenesis and vascular endothelial cell function [4,5].

Features

- Designed to assay compounds for their ability to modulate internalization of S1P$_3$
- 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

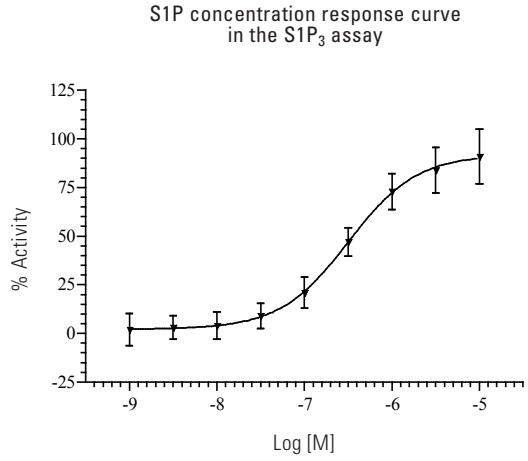

Figure 2. Concentration response curve in the S1P$_3$ agonist assay. S1P concentration response curve in the S1P$_3$ agonist Redistribution assay (n = 16). The EC_{50} value of S1P is 300 nM. Concentration response was measured in 9 point half log dilution series. Cells were incubated with S1P for 60 min. Cells were then fixed and internalization was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (10 µM S1P) 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

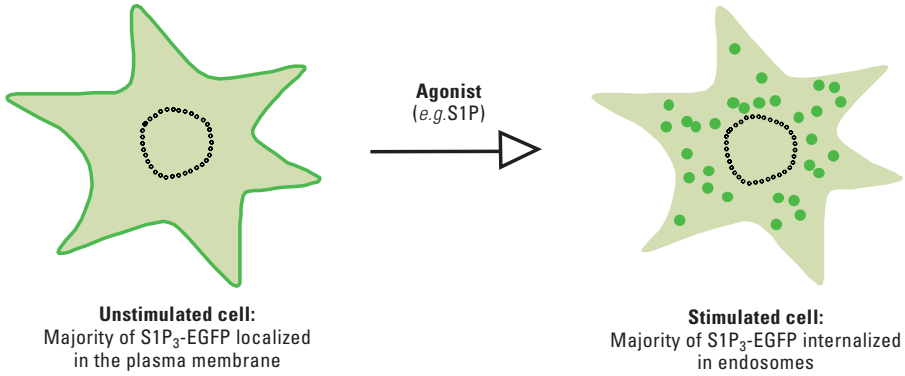

Figure 3. Illustration of the $S1P_3$ translocation event.

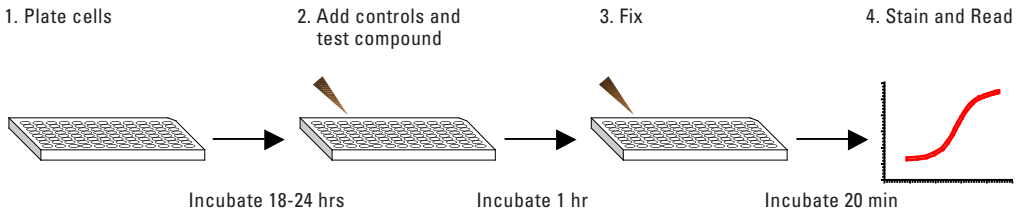

Figure 4. The $S1P_3$ Redistribution assay is very easy and fast to perform.

Thermo Scientific $S1P_3$ Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human $S1P_3$ receptor fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists of $S1P_3$ translocation by monitoring the internalization of membrane-localized $S1P_3$-EGFP fusion protein. In order to differentiate between $S1P_1$ and $S1P_3$ activation, this assay can be performed in conjunction with the $S1P_1$ Redistribution assay (see related products). The $S1P_3$ assay is validated with an average Z' = 0.58 ± 0.07 and is suitable for profiling applications.

Imaging

The translocation of $S1P_3$-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 20x objective or higher magnification. The primary output in the $S1P_3$ Redistribution assay is the formation 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 VTI

This assay has been validated on the Cellomics Arrayscan VTI using a 20x objective (0.63X coupler), High Resolution images, 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.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
095_01	$S1P_3$ 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	CRYOREDI
067_01	CXCR4 Redistribution Assay	U2OS	•	•	
094_01	GRPR Redistribution Assay	U2OS	•	•	
054_01	MCH1 Redistribution Assay	U2OS	•		
039_01	$S1P_1$ Redistribution Assay	U2OS	•	•	•
093_01	CRTH2 Redistribution Assay	U2OS	•	•	
086_01	M1 Redistribution Assay	U2OS	•		
075_01	M2 Redistribution Assay	U2OS	•	•	
076_01	M3 Redistribution Assay	U2OS	•	•	
057_01	MC4 Redistribution Assay	U2OS	•		
053_01	FSHR Redistribution Assay	U2OS	•	•	
051_01	CB1 Redistribution Assay	U2OS	•	•	•
061_01	CB2 Redistribution Assay	U2OS	•		•
097_02	GLP1R Redistribution Assay	U2OS	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – 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	•	•	
8401501	Cellomics Transcription Factor: Phospho-CREB HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404701	Cellomics PKA and Phospho-CREB Activation Kit HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404301	Cellomics PKA 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

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4. Lin CI et al., *Biochem Biophys Res Commun*, 355 (4), 895-901, 2007.
5. Lee MJ et al., *Cell.*;99(3), 301-12, 1999

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