

Thermo Scientific S1P₁ 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 S1P₁-EGFP stimulated with S1P. Cells were untreated (vehicle control, left panel) or treated with 5 μM S1P (right panel). Arrows indicate S1P-induced S1P₁ internalization detected by the image analysis algorithm.

Thermo Scientific S1P₁ 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]. There are five known G protein-coupled S1P receptors in mammals. Binding of S1P to the S1P₁ receptor activates G α i and G α o resulting in adenylate cyclase inhibition, phospholipase C activation, Ca²⁺ mobilization, Ras-Erk activation and PI3 kinase activation [3]. Activation of S1P₁ also results in GPCR kinase 2 (GRK2)-dependent receptor internalization, followed by recycling of the receptor back to the plasma membrane.

Features

- Designed to assay compounds for their ability to modulate internalization of S1P₁
- 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

S1P concentration response curve
in the S1P₁ assay

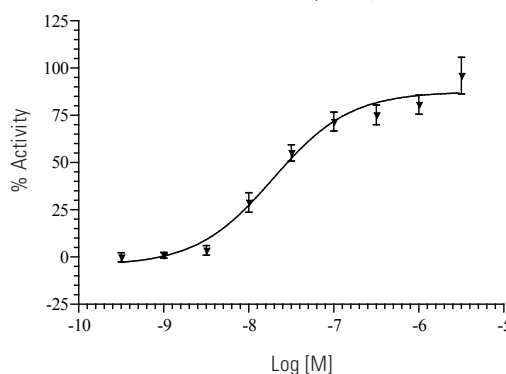


Figure 2. Concentration response curve in the S1P₁ agonist assay. S1P concentration response curve in the S1P₁ agonist Redistribution assay (n = 4). The EC₅₀ value of S1P is 26.9 nM. Concentration response was measured in 9 point half log dilution series. Cells were incubated with S1P for 60 min. Cells were then fixed and internalization was measured using the Cellomics ArrayScan V[®] Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (5 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

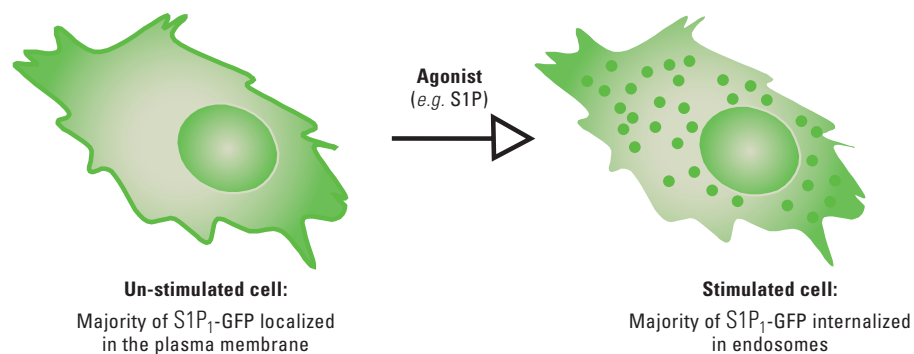


Figure 3. Illustration of the S1P₁ translocation event.

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Assay Details

Recombinant U2OS cells stably expressing human S1P₁ receptor fused to the N-terminus of enhanced green fluorescent protein (EGFP). The S1P₁ agonist Redistribution assay is designed to screen for agonists of S1P₁ translocation by monitoring the internalization of a membrane-localized S1P₁-EGFP fusion protein to endosomes. The EC₅₀ value of S1P is ~25 nM in the assay [1,4] and ligands/compounds are assayed for their ability to induce S1P₁ internalization. The S1P₁ assay is validated with an average Z' = 0.83 ± 0.03, suitable for both screening and profiling applications.

Imaging

The translocation of S1P₁ 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 S1P₁ 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 V™

This assay has been developed on the Cellomics ArrayScan V™ using a 10x 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 150 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

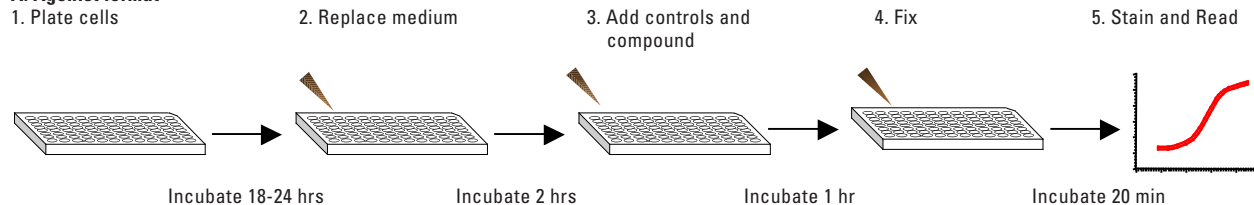
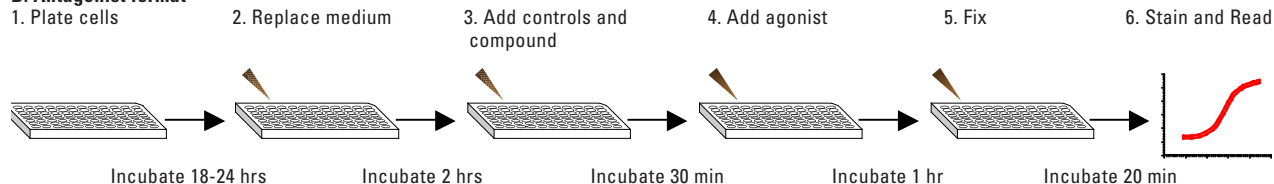
A. Agonist format**B. Antagonist format**

Figure 4. The S1P₁ Redistribution assay is very easy and fast to perform in both agonist and antagonist format.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
039_01	S1P ₁ 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
095_01	S1P ₃ :Redistribution Assay	U2OS	•		
067_01	S1P ₁ :PKA GPCR activation Redistribution Assay	CHO-K1	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
8401401	Cellomics Transcription Factors: HIF-1 alpha, Phospho-CREB, FOXO3a HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404701	Cellomics PKA and Phospho-CREB 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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2. Kostenis E, *J Cell Biochem.*, 92, 923-936, 2004.
3. Okamoto H et al., *J Biol Chem.*, 273, 27104-27110, 1998.
4. Jo E et al., *Chem Biol.*, 12, 703-715, 2005.

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