

Thermo Scientific AT₁R 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.

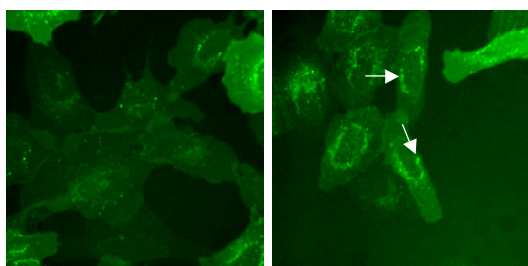


Figure 1. Internalization of AT₁R-EGFP. Cells were treated with 500 nM angiotensin I for 30 min (right panel) or untreated (DMSO control, left panel). Arrows indicate the AT₁R internalization detected by the image analysis algorithm.

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The renin-angiotensin system is a central component of the physiological and pathological responses of the cardiovascular system. Its primary effector hormone, angiotensin, activates a wide spectrum of signaling responses via the AT₁ receptor (AT₁R) thereby mediating its immediate physiological effects of vasoconstriction and blood pressure regulation. It is also implicated in inflammation, endothelial dysfunction, atherosclerosis, hypertension, and congestive heart failure. The AT₁R is a member of the superfamily of G protein-coupled receptors (GPCRs), and angiotensin-induced AT₁R activation via Gq/11 stimulates phospholipases A₂, C, and D. This leads to activation of inositol trisphosphate/Ca²⁺ signaling, protein kinase C isoforms, MAPKs, and tyrosine kinases as well as scaffold proteins. Angiotensin activation of the AT₁R causes rapid phosphorylation, desensitization, and endocytosis of the AT₁R with subsequent intracellular processing and recycling to the cell membrane.

In addition to the established therapeutic actions of angiotensin in hypertension, the actions of angiotensin have led to novel clinical applications of angiotensin-converting enzyme (ACE) inhibitors and AT₁R blockers within the fields of cancer, diabetes, and metabolic diseases. Antagonists of AT₁R internalization are likely to be relevant in the treatment of hypertension and related diseases [1-3].

Features

- Designed to assay compounds for their ability to modulate internalization of AT₁R
- 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

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

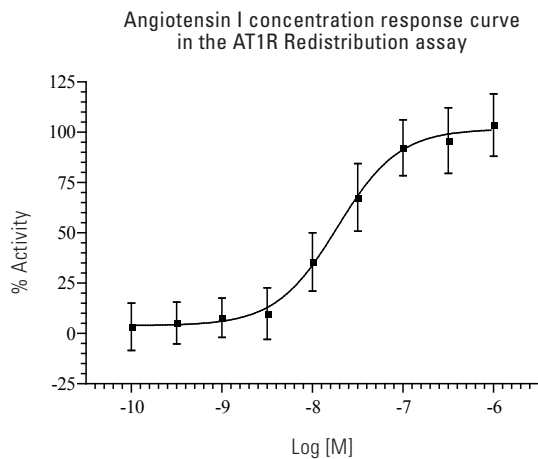


Figure 2. Concentration response curve in the AT1R assay: The EC_{50} of angiotensin I is approximately 20 nM ($n = 32$). Cells were treated with angiotensin I for 30 min. Cells were then fixed and receptor internalization was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (500 nM angiotensin I) and negative control (0.25% DMSO).

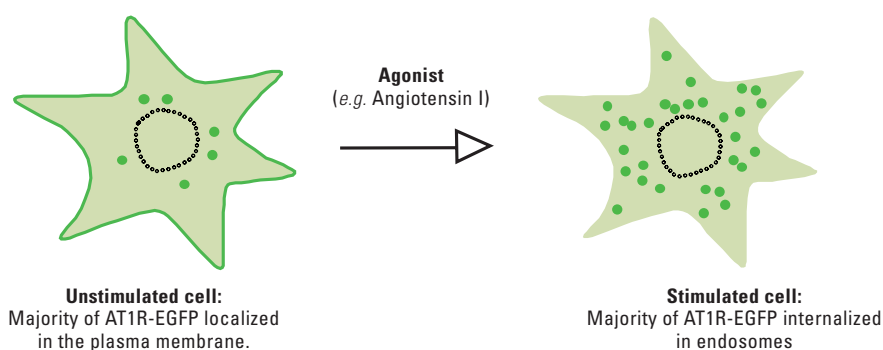


Figure 3. Illustration of the AT1R translocation event.

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Assay Details

Recombinant U2OS cells stably expressing human angiotensin II Type-1 receptor (AT1R) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists causing internalization of AT1R. Angiotensin I is used as reference compound. The AT1R assay is validated with an average 0.43 ± 0.11 , suitable for both profiling and screening applications.

Imaging

The translocation of AT1R-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 10-20x objective or higher magnification. The primary output in the AT1R 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^{TI}

This assay has been validated on the Cellomics ArrayScan V^{TI} 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 500 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

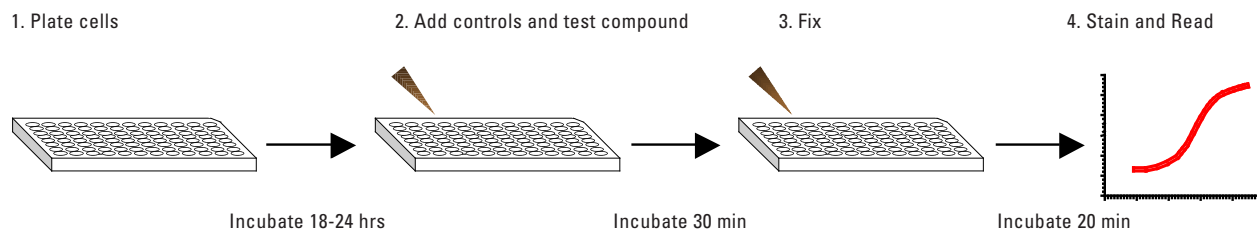


Figure 4. The AT1R Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
071_01	AT1R Redistribution Assay	U20S	•	•	

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	U20S	•	•	
094_01	GRPR Redistribution Assay	U20S	•	•	
054_01	MCH1 Redistribution Assay	U20S	•		
039_01	S1P ₁ Redistribution Assay	U20S	•	•	•
095_01	S1P ₃ Redistribution Assay	U20S	•		•
086_01	M1 Redistribution Assay	U20S	•		
075_01	M2 Redistribution Assay	U20S	•	•	
076_01	M3 Redistribution Assay	U20S	•	•	
053_01	FSHR Redistribution Assay	U20S	•	•	
093_01	CRT2 Redistribution Assay	U20S	•	•	
051_01	CB1 Redistribution Assay	U20S	•	•	•
061_01	CB2 Redistribution Assay	U20S	•		•
097_02	GLP1R Redistribution Assay	U20S	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U20S	•	•	
K0100111	Cellomics NFAT-1 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100071	Cellomics ERK MAPK 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

- de Gasparo M et al. *Pharmacol Rev.*, 52, 415-472, 2000.
- Hunyady L & Catt KJ, *Mol Endocrinol.*, 20, 953-970, 2006.
- Mehta PK & Griendling KK, *Am J Physiol Cell Physiol.*, 292:C82-97, 2007.

