

Thermo Scientific PXR 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.

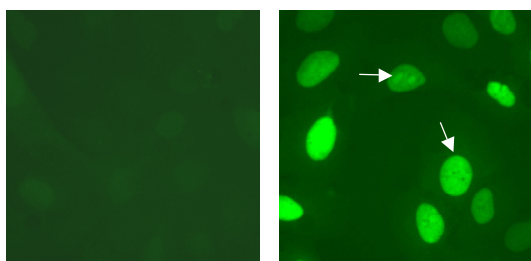


Figure 1. Accumulation of EGFP-PXR in response to anisomycin. Cells were treated with 300 nM anisomycin for 24 hrs (right panel) or untreated (DMSO control, left panel). Arrows indicate nuclear accumulation of PXR detected by the image analysis algorithm.

Thermo Scientific PXR Redistribution Assay

The pregnane X receptor (PXR), also known as the steroid and xenobiotic receptor (SXR) or pregnenolone-activated receptor (PAR), is a member of the nuclear receptor family of ligand-activated transcription factors, and a key transcriptional regulator of the expression of metabolizing and detoxifying enzymes involved in endobiotic and xenobiotic metabolism. PXR is expressed predominantly in liver and intestine and is activated by a variety of structurally distinct ligands, triggering up-regulation of genes central to drug metabolism and drug efflux transporter genes, thereby increasing metabolism and excretion of therapeutic agents as well as in drug-drug interactions. In addition, PXR is also activated by a variety of endogenous ligands including pregnanes, bile acids, hormones, and dietary vitamins. Genes regulated by PXR include phase I (e.g. cytochrome P450 (CYP450)) and phase II drug-metabolizing enzymes as well as drug transporters such as ABC transporters and multi drug resistance proteins MDR1 and MDR2. Induction of drug efflux transporters

and drug-metabolizing proteins is a major underlying mechanism in the development of drug resistance in cancer. The PXR Redistribution assay makes it possible to evaluate PXR-mediated drug metabolism of test compounds at an early stage during drug discovery as well as being an early indicator of drug toxicity [1-4].

Features

- Designed to assay compounds for their ability to induce nuclear accumulation of PXR
- 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

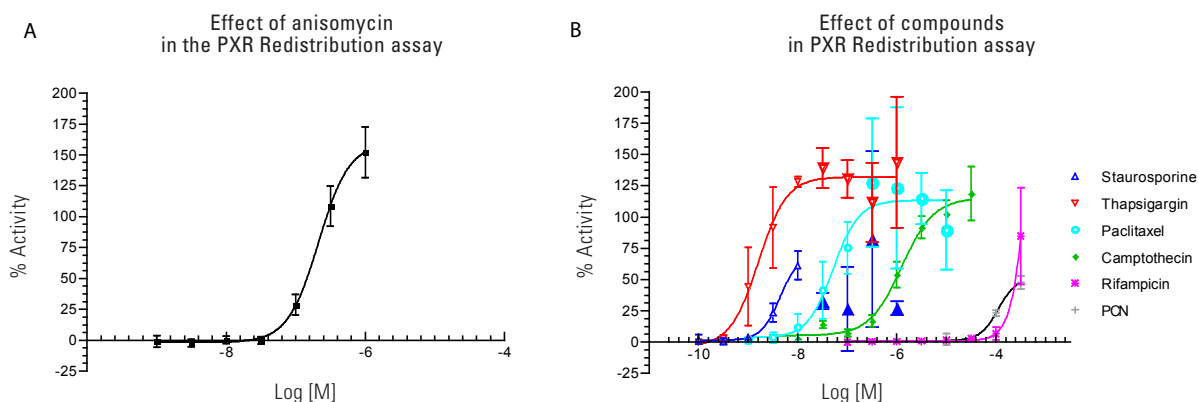


Figure 2. Concentration response of reference compounds in the PXR Redistribution Assay. A) Concentration response was measured in 9 point half log dilution series. Cells were incubated with anisomycin for 24 h. Cells were then fixed and the translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the RedistributionV3 BioApplication. % activity was calculated relative to the positive (300 nM anisomycin) and negative control (0.25% DMSO). The EC₅₀ of anisomycin is approximately 200 nM, n = 12. **B)** A panel of compounds with known toxic profile was profiled in the PXR Redistribution assay. Data points in bold indicate concentrations of compound with tox effects on cell morphology.

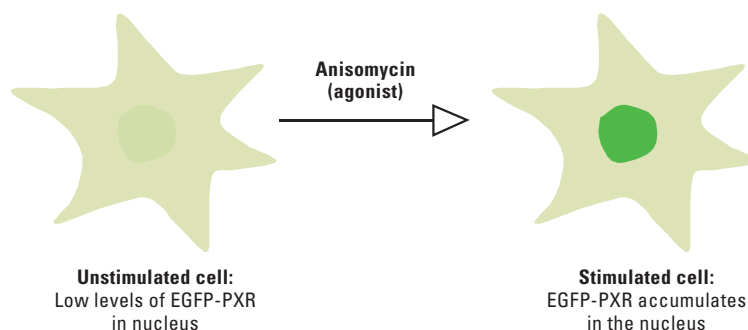


Figure 3. Illustration of the PXR translocation event.

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Assay Details

Recombinant U2OS cells stably expressing human Pregnane X Receptor (PXR) fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to identify PXR activators by monitoring the accumulation of EGFP-PXR fusion protein in the nucleus. Anisomycin is used as the reference agonist. The PXR assay is validated with an average Z' = 0.62 ± 0.12, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-PXR 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 PXR Redistribution assay is the translocation and accumulation of EGFP-PXR in the nucleus. The data analysis should therefore report an output relating to the GFP fluorescence intensities in the nucleus and 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 Redistribution V3 BioApplication. The output used was MEAN_CircAvgInten. 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 Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2 and ColocalizationV3.

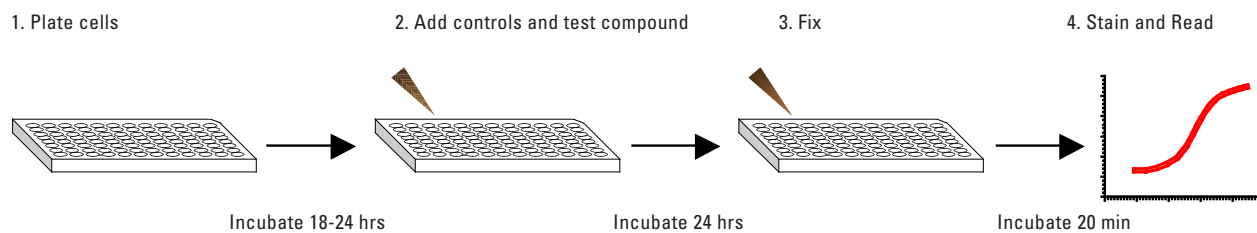


Figure 4. The PXR Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
077_01	PXR 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
084_01	ATF6 Redistribution Assay	U2OS	•	•	•
042_01	Rad51 Redistribution Assay	SW480	•	•	•
8408102	Cellomics Cytotoxicity 3 Multiparameter HCS Reagent Kit	Antibody- and dye-based reagent kit			
8408001	Cellomics Multiparameter Cell Death Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0400011	Cellomics Multiparameter Apoptosis 1 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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