

Thermo Scientific PR (Progesterone Receptor) 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.

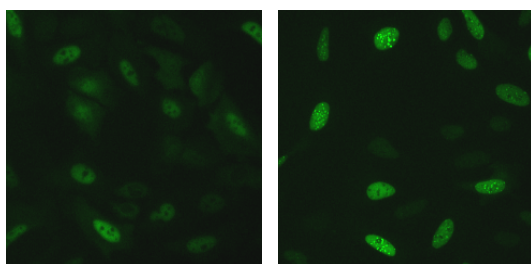


Figure 1. Nuclear translocation and foci formation of EGFP-PR. Cells were treated with 30 nM progesterone for 30 min (right panel) or untreated (DMSO control, left panel). This causes EGFP-PR to disappear from the cytoplasm and accumulate in the nucleus; this altered distribution is detected by the image analysis algorithm.

Thermo Scientific PR (Progesterone Receptor) Redistribution Assay

Progesterone is a female gonadal steroid hormone synthesized in the ovary. The progesterone receptor (PR) that specifically binds progesterone is member of the steroid receptor superfamily of nuclear receptors. The A form (PR-A) is a 164-amino acid N-terminally truncated version of the B form (PR-B). Unliganded PR is localized in the cytoplasm as well in the nucleus. Hormone binding to PR induces a conformational shift followed by dimerization into a complex that accumulates in the nucleus. In the nucleus PR activates and represses transcription of a range of target genes. Mice lacking PR exhibit reproductive abnormalities, incomplete mammary gland development, and impaired thymic function and sexual behavior [1]. Clinically, progestational agents are used in oral contraceptives, menopausal hormone

replacement, and treatment of endometrial hyperplasia and breast cancer. The PR redistribution assay is based on human PR-B fused to EGFP and monitors translocation of EGFP-PR from the cytoplasm to the nucleus using progesterone as a reference compound. In addition to nuclear translocation, progesterone and other ligands induce accumulation of EGFP-PR in nuclear foci.

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of the Progesterone Receptor
- 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

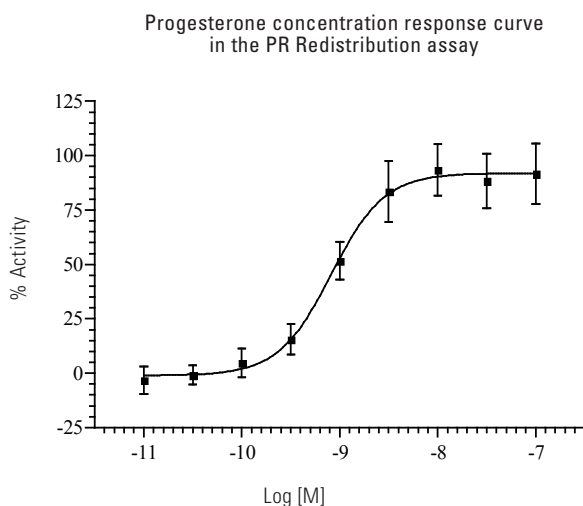


Figure 2. Concentration response curve in the PR assay: Progesterone concentration response in the PR agonist assay (n = 16). The EC_{50} is approximately 0.8 nM. Concentration response was measured in 9 point half log dilution series. Cells were treated with progesterone for 30 min. Cells were then fixed and nuclear foci formation was measured using the Cellomics ArrayScan V^{TI} Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (30 nM progesterone) and negative control (0.25% DMSO).

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Assay Details

Recombinant U2OS cells stably expressing human progesterone receptor (PR) fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to test for compounds that induce PR translocation from the cytoplasm to the nucleus. Progesterone is used as reference compound. Compounds causing nuclear translocation of PR could be acting either as agonists or antagonists, or could be general nuclear import activators/nuclear export inhibitors. Compounds inducing nuclear accumulation of PR can be counter-screened for general export inhibitor characteristics using the Rev1 Redistribution assay (see related products). The PR assay is validated with an average $Z' = 0.43 \pm 0.05$, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-PR 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 Progesterone Redistribution assay is the translocation from cytoplasm to nucleus of EGFP-PR. 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_CircRingAvgIntenRatioLog (Log of the ratio of average fluorescence intensities of nucleus and cytoplasm (well average)). The minimally acceptable number of cells used for image analysis in each well was set to 100 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

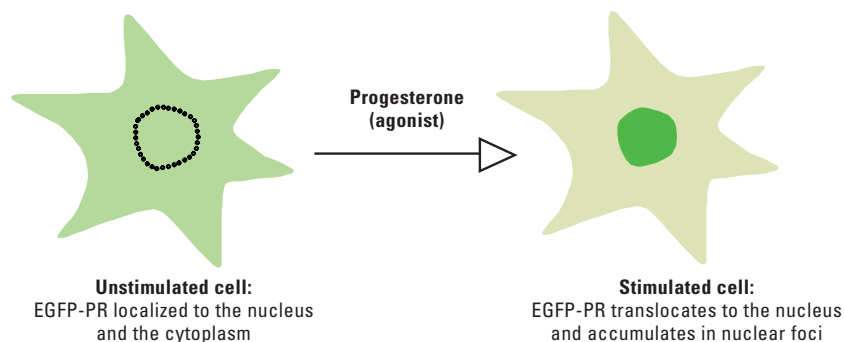


Figure 3. Illustration of the PR translocation event.

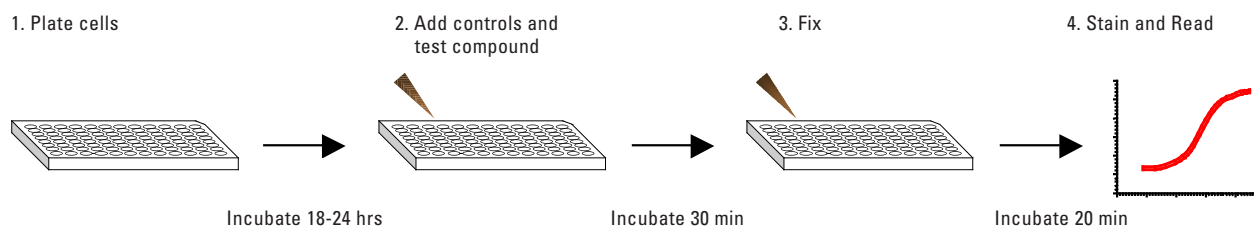


Figure 4. The PR Redistribution assay is very easy and fast to perform

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
064_01	PR 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
043_01	AR Redistribution Assay	U2OS	•	•	•
060_01	VDR Redistribution Assay	U2OS	•	•	•
056_01	ER α Redistribution Assay	U2OS	•	•	•
063_01	ER β Redistribution Assay	U2OS	•	•	•
014_01	GR Redistribution Assay	U2OS	•	•	•
077_01	PXR Redistribution Assay	U2OS	•	•	•
062_01	REV Redistribution Assay	U2OS	•	•	
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

- Lydon J et al. *Genes Dev.* 9:2266-2278, 1995.
- Li H. et al. *Pharmaceutical Research.* 20(10):1574-1580, 2003.
- Zhi L. et al. *J. Med. Chem.* 46: 4104-12, 2003

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