

Thermo Scientific GR 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.

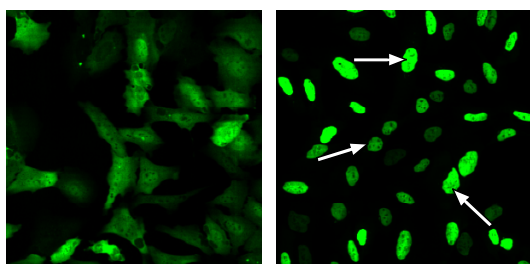


Figure 1. Nuclear translocation of EGFP-GR. Cells were treated without (DMSO control, left panel) or with (right panel) 100 nM dexamethasone for 2 hours. Arrows indicate the nuclear translocation of EGFP-GR detected by the image analysis algorithm.

Thermo Scientific GR Redistribution Assay

Glucocorticoids are the most potent and effective drugs for preventing and suppressing inflammation caused by mechanical, chemical, infectious, and immunological stimuli. They exert their activity through binding to the glucocorticoid receptor (GR) resulting in either activation or repression of a large set of glucocorticoid responsive genes. GR is a ligand-dependent transcription factor belonging to the superfamily of steroid hormone receptors, which includes the mineralocorticoid, thyroid hormone, sex hormones, retinoic acid, and vitamin D receptors [1]. The GR receptor contains a DNA binding site, a hormone-independent activation function (AF1) domain in the N-terminus, which is associated with transcriptional activity and binding to co-activators and transcription factors, and a hormone-dependent AF2 domain in the C-terminus responsible for hormone binding as well as binding of co-activators. In the inactive state, the GR is found in the cytoplasm in complex with heat

shock proteins. Upon ligand binding, the GR translocates to the nucleus, dimerizes, and acts as a transcription factor to regulate the expression of its target genes. GR agonists such as dexamethasone induce nuclear translocation and allow the receptor to bind co-activators with intrinsic histone acetylase and methylase activities, such as proteins from the SRC-1, p300/CBP, and PRMT families, whereas GR antagonists, which also induce nuclear translocation of GR, result in the recruitment of histone deacetylases through co-repressors of the N-CoR/SMRT family [2].

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of GR
- 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

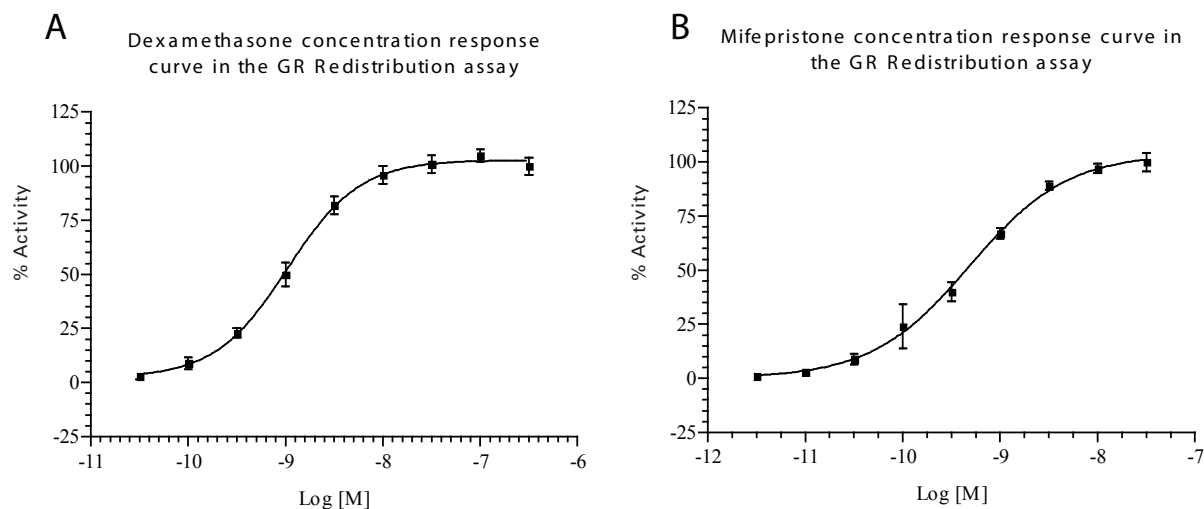


Figure 2. Concentration response curves in the GR assay: A) Dexamethasone concentration response in the GR assay (n=6). The EC_{50} is approximately 1 nM. B) Mifepristone concentration response in the GR assay (n=3). The EC_{50} is approximately 0.5 nM. The concentration response was measured in 9 point half log dilution series. Cells were treated with compound for 2 hrs. Cells were then fixed and imaging was performed using an image algorithm detecting nuclear translocation. % activity was calculated relative to the positive (100 nM dexamethasone) and negative control (0.25% DMSO)

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Assay Details

Recombinant U2OS cells stably expressing human glucocorticoid receptor (GR) fused to the C-terminus of enhanced green fluorescent protein (EGFP). The GR Redistribution Assay is designed to assay for compounds inducing GR translocation by monitoring the translocation of a GFP-GR fusion protein from the cytoplasm to the nucleus. GR nuclear translocation can be promoted by the agonist dexamethasone [3,4], which is used as reference compound in this assay. Compounds causing nuclear translocation of GR could be acting either as agonists or antagonists, or could be general nuclear import activators/nuclear export inhibitors. Compounds inducing nuclear accumulation of GR can be counter-screened for general export inhibitor characteristics using the Rev1 Redistribution Assay (see related products). The GR assay is validated with an average $Z' = 0.84 \pm 0.03$, suitable for both screening and profiling applications.

Imaging

The translocation of GR 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 GR Redistribution assay is the translocation from

cytoplasm to nucleus of EGFP-GR. 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™

This assay has been validated on the Cellomics Arrayscan V™ 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 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2 and ColocalizationV3.

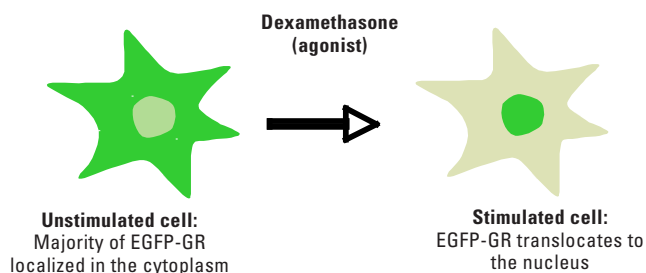


Figure 3. Illustration of the GR translocation event.

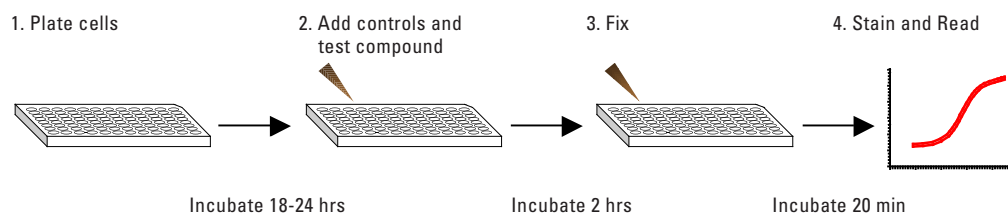


Figure 4. The GR Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
014_01	GR 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	•	•	•
064_01	PR Redistribution Assay	U2OS	•	•	•
077_01	PXR Redistribution Assay	U2OS	•	•	•
062_01	REV Redistribution Assay	U2OS	•	•	
8401401	Cellomics HIF-1 alpha, Phospho-CREB, FOXO3a HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404601	Cellomics Cell Cycle Kit I HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401101	Cellomics BrdU and Ki67 Cell Proliferation 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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3. Hardt FD, et al. *Lancet.* 2, 255-257, 1959.
4. Levinson BB et al. *Science* 175, 189-190, 1972.

