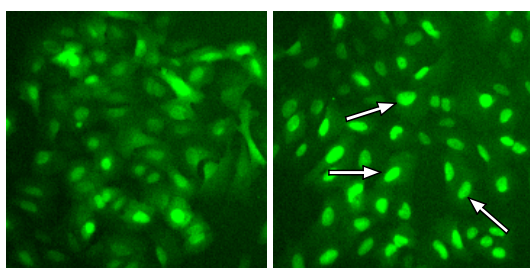


## Thermo Scientific VDR (Vitamin D Receptor) Redistribution<sup>®</sup> Assay

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-VDR.** Cells were treated with 300 nM Vitamin D<sub>3</sub> for 30 min (right panel) or untreated (DMSO control, left panel). Arrows indicate the nuclear localization of EGFP-VDR detected by the image analysis algorithm.

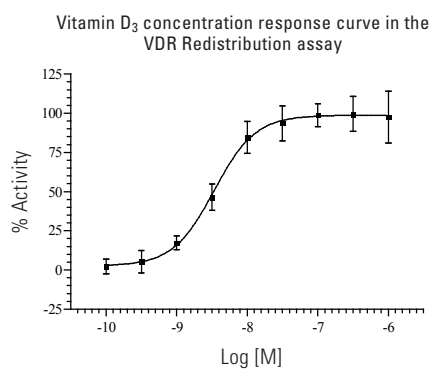
### Thermo Scientific VDR (Vitamin D Receptor) Redistribution Assay

The vitamin D receptor (VDR) is a member of the steroid receptor (nuclear hormone receptor) family and mediates gene expression in a vitamin D/calcitriol dependent fashion. Calcitriol, the active form of vitamin D, regulates various aspects of calcium metabolism and bone homeostasis. In addition, vitamin D and analogs thereof also possess potent differentiative, anti-proliferative, and immunomodulatory activities, and is currently used clinically to treat psoriasis. VDR functions as a sequence-specific transcription factor as a hetero-dimer with the Retinoid X Receptor (RXR). In the absence of ligand, VDR complexes with co-repressor proteins to repress target genes. Binding of ligand to the receptor induces a conformational shift in the receptor and

allows the exchange of co-repressors with co-activators and activation of target genes [1,2].

#### Features

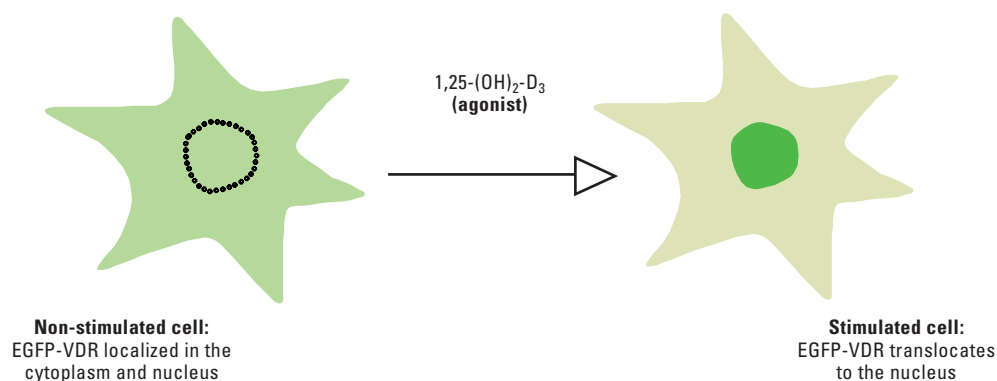
- Designed to assay compounds for their ability to modulate nuclear translocation of the Vitamin D Receptor
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications



**Figure 2. Vitamin D<sub>3</sub> concentration response in the VDR assay.** Concentration response was measured in 9 point half log dilution series (n = 16). The EC<sub>50</sub> is approximately 3 nM. Cells were treated with Vitamin D<sub>3</sub> for 30 min. Cells were then fixed and nuclear translocation was measured using the Cellomics ArrayScan V<sup>™</sup> Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (300 nM Vitamin D<sub>3</sub>) 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 VDR translocation event.

## Thermo Scientific VDR (Vitamin D Receptor) Redistribution® Assay

### Assay Details

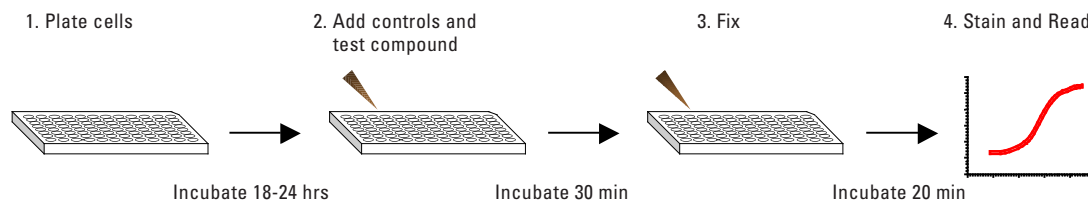
Recombinant U2OS cells stably expressing human Vitamin D receptor (VDR) fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to test for compounds that induce VDR translocation from the cytoplasm to the nucleus. The reference ligand is 1,25-(OH)<sub>2</sub>-D<sub>3</sub> (calcitriol). Similar Redistribution assays are available for the androgen receptor, estrogen receptor, progesterone receptor, and glucocorticoid receptor (see related products). The VDR assay is validated with an average  $Z' = 0.56 \pm 0.07$ , suitable for both screening and profiling applications.

### Imaging

The translocation of EGFP-VDR 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 VDR Redistribution assay is the translocation from cytoplasm to nucleus of EGFP-VDR. 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<sup>ti</sup>

This assay has been validated on the Cellomics ArrayScan V<sup>ti</sup> 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 150 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.



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

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
060_01	VDR 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	•	•	•
064_01	PR Redistribution Assay	U2OS	•	•	•
056_01	ER $\alpha$ Redistribution Assay	U2OS	•	•	•
063_01	ER $\beta$ 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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

### References

1. Racz et al: *JBC* 1999; 274: 19352-60.
2. Campbell MJ, Adorini L. *Expert Opin Ther Targets*. 2006;10(5):735-48.

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