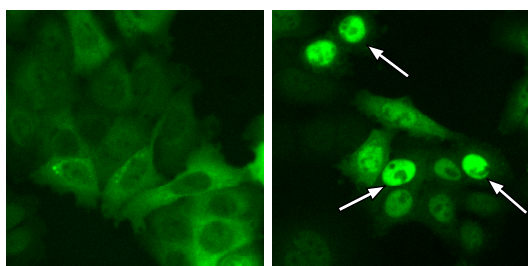


## Thermo Scientific HDAC5 Redistribution<sup>®</sup> Assay

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. Translocation of EGFP-HDAC5 in response to prazosin.** Cells were treated with 50  $\mu$ M phenylephrine (control, left panel) or 50  $\mu$ M phenylephrine + 100 nM prazosin (right panel). Arrows indicate cytoplasm to nucleus translocation detected by the image analysis algorithm.

### Thermo Scientific HDAC5 Redistribution Assay

Class II histone deacetylases HDAC4,5,7, and 9 shuttle between the cytoplasm and the nucleus in a signal-dependent manner. In the nucleus, they bind to transcriptional regulators such as the MEF2 family and repress transcription from target promoters. In cardiomyocytes, HDAC5 and HDAC9 perform similar functions by restricting a MEF2-dependent fetal transcription program involved in cardiac hypertrophy, a leading cause of heart failure.

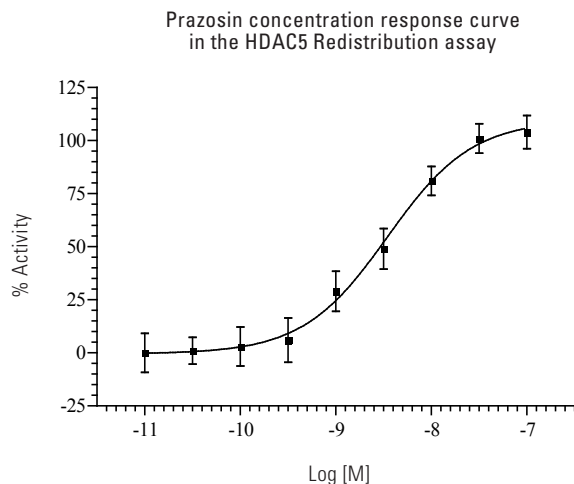
Hypertrophic agonists such as serotonin and phenylephrine stimulate PKD-dependent phosphorylation of HDAC5 and HDAC9 through a PKC-dependent pathway. Phosphorylation leads to nuclear exclusion of the HDAC, which is the dominant way of regulating HDAC5/9 activity in the cell. Compounds that induce nuclear retention of HDAC5/9 are therefore relevant drug candidates for the treatment of cardiac hypertrophy and other hypertrophic diseases [1-3].

### Features

- Designed to assay compounds for their ability to modulate nuclear translocation of HDAC5
- 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. Prazosin concentration response curve in the HDAC5 Redistribution assay.** Concentration response was measured in 9 point half log dilution series of prazosin. Cells were incubated with prazosin for 2 h. Cells were then fixed and the nucleus to cytoplasm translocation was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the RedistributionV3 BioApplication. % activity was calculated relative to the positive (100 nM prazosin) and negative control (0.25% DMSO). The EC<sub>50</sub> of prazosin is ~3.5 nM.

## Thermo Scientific HDAC5 Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant SW480 cells stably expressing human HDAC5 fused to the C-terminus of enhanced green fluorescent protein (EGFP) and  $\alpha$ 1B adrenergic receptor. SW480 cells are adherent epithelial cells derived from human colorectal adenocarcinoma. In this assay the  $\alpha$ 1B adrenergic receptor and HDAC5 are co-expressed. Activation of the  $\alpha$ 1B adrenergic receptor by phenylephrine results in export of HDAC5 from the nucleus. The assay is designed to identify antagonists of the HDAC5 nuclear export, using prazosin as reference compound. The HDAC5 assay is validated with an average  $Z' = 0.59 \pm 0.06$ , suitable for both screening and profiling applications.

### Imaging

The translocation of EGFP-HDAC5 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 20x objective or higher magnification. The primary output in the HDAC5 Redistribution assay

is the translocation of EGFP-HDAC5 between the cytoplasm and 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<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 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

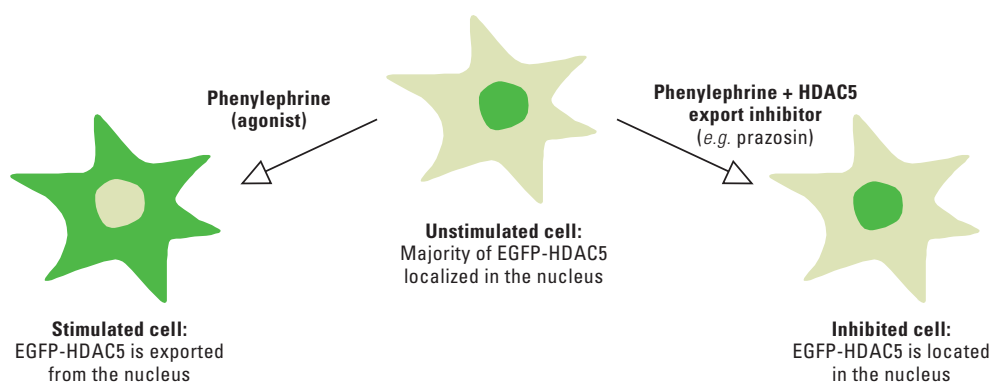


Figure 3. Illustration of the HDAC5 translocation event.

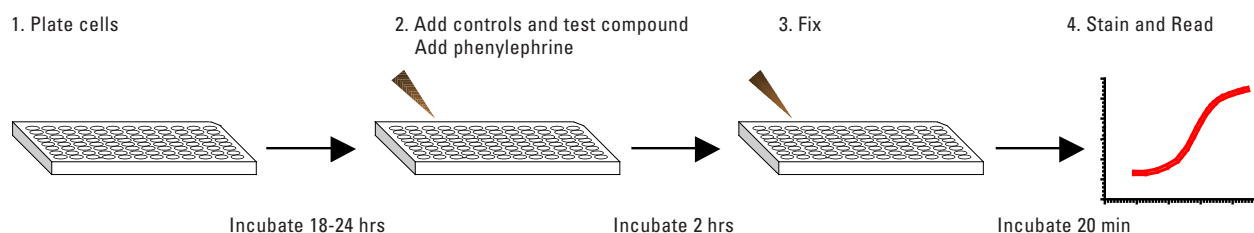


Figure 4. The HDAC5 Redistribution assay is very easy and fast to perform.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
069_01	HDAC5 Redistribution Assay	SW480	•	•	

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
062_01	REV Redistribution Assay	U2OS	•	•	
K0900011	Cellomics PKC alpha 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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

### References

1. Chang et al. *Mol. Cell. Biol.* 2004; 24: 8467-76
2. Vega et al. *Mol. Cell. Biol.* 2004; 24: 8374-8385
3. Harrison et al. *Mol. Cell. Biol.* 2006; 26: 3875-3888.

