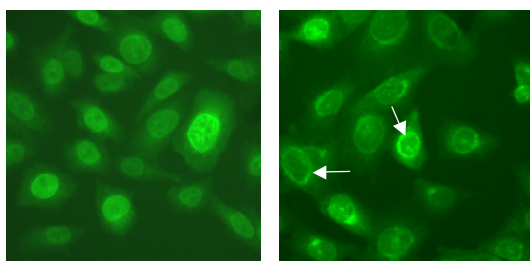


## Thermo Scientific 5-LOX Redistribution<sup>®</sup> Assay

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 5-LOX-EGFP to the nuclear envelope.** Cells were treated with 300 nM A23187 for 5 min (right panel) or untreated (DMSO control, left panel). Arrows indicate the nuclear envelope localization detected by the image analysis algorithm.

### Thermo Scientific 5-LOX Redistribution Assay

5-Lipoxygenase (5-LOX) is a lipid-peroxidizing enzyme that plays an essential role in the biosynthesis of leukotrienes, which mediate inflammatory and allergic reactions. The key regulatory steps in leukotriene biosynthesis following cell activation include  $\text{Ca}^{2+}$  mobilization and subsequent release of arachidonic acid (AA) from membrane phospholipids by phospholipase A2. In a  $\text{Ca}^{2+}$ - and ATP-dependent reaction, AA is then metabolized by 5-LOX to yield the epoxide intermediate leukotriene A4 (LTA4). This step is dependent upon the interaction of 5-LOX with the nuclear membrane protein 5-lipoxygenase activating protein (FLAP) and requires translocation of 5-LOX to the nuclear envelope. FLAP then presents AA to 5-LOX and thereby increases the catalytic potential of 5-LOX. In addition to being activated by an increase in intracellular  $\text{Ca}^{2+}$  concentration, 5-LOX can be activated by diacylglycerols as well as by phosphorylation by MAPKAP kinase-2 and ERK.

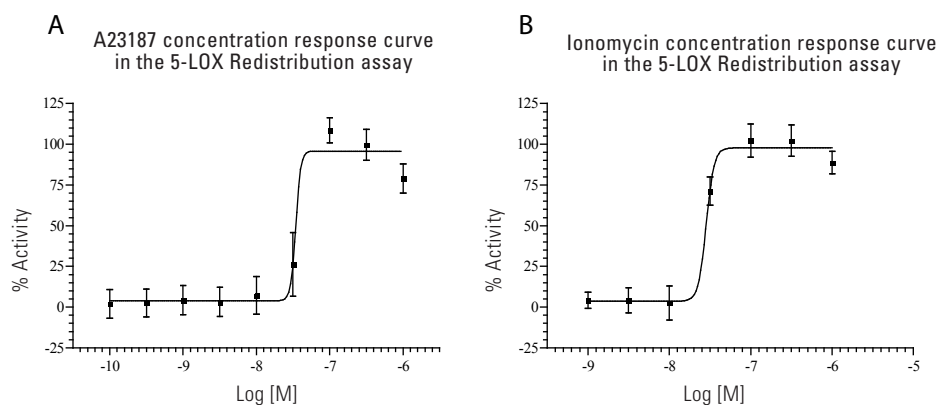
In addition to the classical application of leukotriene synthesis inhibitors in asthma and allergic disorders, leukotriene synthesis inhibitors might be of value for the treatment of cardiovascular diseases and osteoporosis. Moreover, dual 5-LOX/COX inhibitors are potential new drugs to treat inflammation, since they act by blocking the formation of both prostaglandins and leukotrienes. [1-4].

### Features

- Designed to assay compounds for their ability to modulate translocation of 5-LOX to the nuclear envelope
- 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 5-LOX assay:** **A)** A23187 concentration response. The  $EC_{50}$  is approximately 35 nM. Concentration response was measured in 9 point half log dilution series ( $n = 16$ ). Cells were treated with A23187 for 5 min. Cells were then fixed and translocation to the nuclear envelope was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (300 nM A23187) and negative control (0.25% DMSO). **B)** Ionomycin concentration response. The  $EC_{50}$  is approximately 30 nM. Concentration response was measured in 7 point half log dilution series ( $n = 16$ ). Cell treatment and image analysis was performed as in A).



**Figure 3.** Illustration of the 5-LOX translocation event.

## Thermo Scientific 5-LOX Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant CHOHR cells stably expressing human 5-Lipoxygenase (5-LOX) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists of 5-LOX by monitoring the translocation of 5-LOX to the nuclear envelope. The ionophore A23187 (also known as calicimycin) is used as reference compound. The 5-LOX assay is validated with an average  $Z' = 0.54 \pm 0.07$ , suitable for both screening and profiling applications.

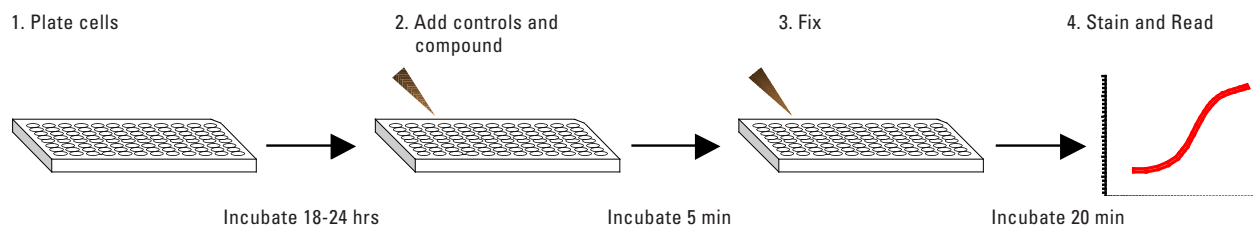
### Imaging

The translocation of 5-LOX-EGFP 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 5-LOX Redistribution assay is the translocation from the nucleus to the nuclear envelope of 5-LOX-EGFP. The data analysis should therefore report an output relating to the GFP fluorescence intensities in the nucleus and the nuclear envelope.

### 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 20x 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 400 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.



**Figure 4.** The 5-LOX Redistribution assay is very easy and fast to perform.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
083_01	5-LOX Redistribution Assay	CHO	•	•	

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
099_01	PKC $\epsilon$ Redistribution Assay	U2OS	•	•	
098_01	PKC $\beta$ Redistribution Assay	U2OS	•		
096_01	MARCKS Redistribution Assay	U2OS	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
8401001	Cellomics Oxidative Stress 1 HCS Reagent Kit	Antibody- and dye-based reagent kit			
8407001	Cellomics MnSOD Induction 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			

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