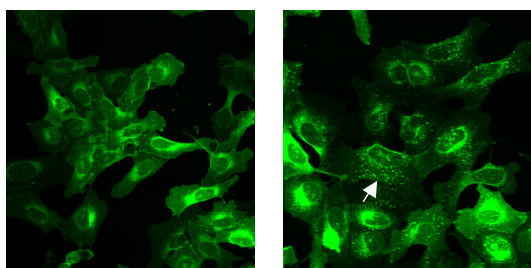


## Thermo Scientific MC4 Redistribution<sup>®</sup> Assay

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. Internalization of MC4-EGFP.** Cells were untreated (DMSO control, left panel) or treated with 10 nM NDP- $\alpha$ -MSH for 2 hr (right panel). Arrow indicates the MC4 internalization detected by the image analysis algorithm.

### Thermo Scientific MC4 Redistribution Assay

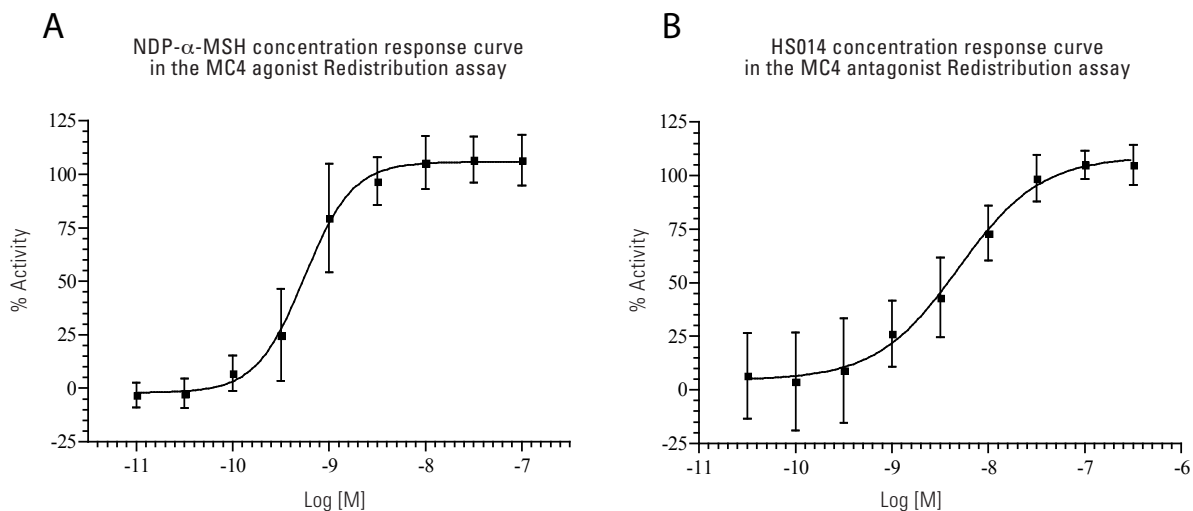
Melanocortins are peptides that regulate eating behavior, metabolism, and possibly also emotional states. The melanocortin receptors are expressed in the central nervous system and are divided into five subtypes (MC1-MC5). They are members of the family of G-protein coupled receptors (GPCRs) and signal through the  $G\alpha_s$  subunit that stimulates production of cAMP by adenylate cyclase. Activation of the MC4 receptor also initiates other regulatory mechanisms such as receptor internalization. The role of MC4 in body weight control is evident from studies in mice, where loss of function mutations are associated with obesity. Moreover, such MC4 mutations are the most common monogenic defect in human obesity. Thus, MC4 is a possible therapeutic target in control of body weight. The natural ligand for MC4 is the alpha-melanocyte-stimulating hormone  $\alpha$ -MSH that is released from synthesizing neurons [1-5].

### Features

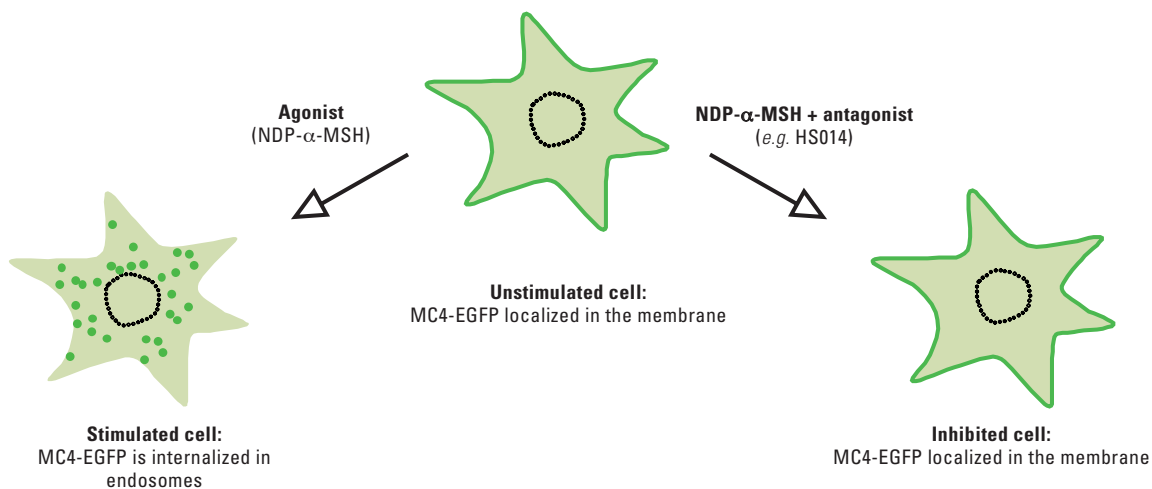
- Designed to assay compounds for their ability to modulate internalization of the MC4 receptor
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

#### Highlights:

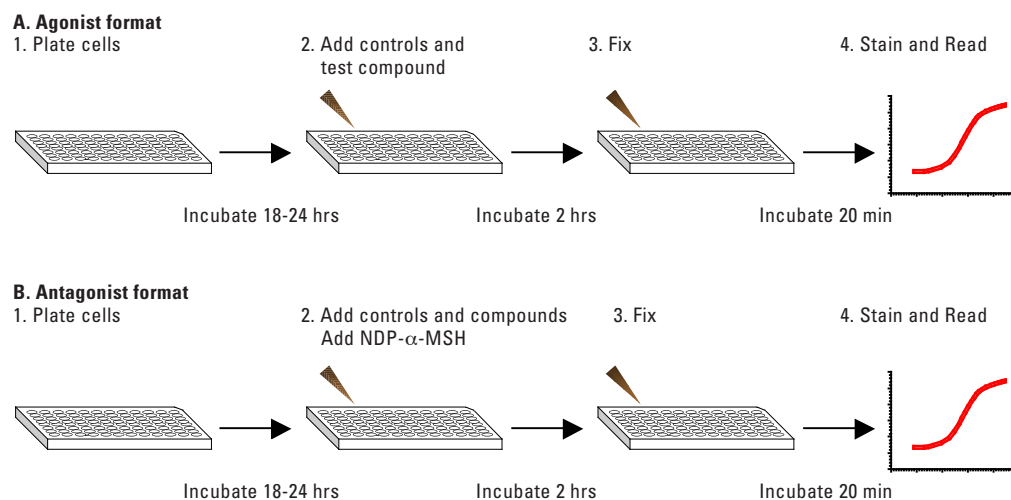
- **Biologically relevant data**  
Compounds tested in a cellular environment
- **Validated**  
Functionally tested cells provided with an optimized assay protocol
- **Easy to use**  
Just plate cells, add compounds, and image



**Figure 2. Concentration response curves in the MC4 assay:** **A)** NDP- $\alpha$ -MSH concentration response in the MC4 agonist assay ( $n = 16$ ). The  $EC_{50}$  is approximately 0.6 nM. Concentration response was measured in 9 point half log dilution series. Cells were treated with NDP- $\alpha$ -MSH for 2 hr. Cells were then fixed and receptor internalization was measured using the IN Cell Analyzer 3000 (GE Healthcare). % activity was calculated relative to the positive (10 nM NDP- $\alpha$ -MSH) and negative control (0.25% DMSO). **B)** HS014 concentration response in the MC4 antagonist assay ( $n = 16$ ). The  $EC_{50}$  is approximately 5 nM. Cells were treated with NDP- $\alpha$ -MSH in the presence of a half log dilution series of HS014 for 2 hr. Cells were then fixed and receptor internalization was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (100 nM HS014) and negative control (0.25% DMSO)



**Figure 3.** Illustration of the MC4 translocation event.



**Figure 4.** The MC4 Redistribution assay is very easy and fast to perform in both agonist and antagonist format.

## Thermo Scientific MC4 Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant U2OS cells stably expressing human melanocortin receptor 4 (MC4) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay monitors internalization of the MC4 receptor from the plasma membrane to endosomes. In the agonist format, a synthetic analog of the natural agonist  $\alpha$ -MSH, NDP- $\alpha$ -MSH, is used as a reference compound. In the antagonist format, a cyclic analog of  $\alpha$ -MSH, HS014, is used as a reference antagonist. The MC4 assay is validated with an average  $Z' = 0.37 \pm 0.15$  and is suitable for profiling applications.

### Imaging

The translocation of EGFP-MC4 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 40x objective or higher magnification. The primary output in the MC4 Redistribution assay is the formation of spots in the cytoplasm. The data analysis should therefore report an output that corresponds to number, area or intensity of spots in the cytoplasm.

### Imaging on Thermo Scientific Cellomics ArrayScan V<sup>™</sup>

This assay has been validated on the Cellomics Arrayscan V<sup>™</sup> using a 40x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. 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 CompartmentalAnalysisV2 and ColocalizationV3.

**Ordering Information**

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
057_01	MC4 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
067_01	CXCR4 Redistribution Assay	U2OS	•	•	
094_01	GRPR Redistribution Assay	U2OS	•	•	
054_01	MCH1 Redistribution Assay	U2OS	•		
039_01	S1P1 Redistribution Assay	U2OS	•	•	•
095_01	S1P3 Redistribution Assay	U2OS	•		•
086_01	M1 Redistribution Assay	U2OS	•		
075_01	M2 Redistribution Assay	U2OS	•	•	
076_01	M3 Redistribution Assay	U2OS	•	•	
053_01	FSHR Redistribution Assay	U2OS	•	•	
093_01	CRTH2 Redistribution Assay	U2OS	•	•	
051_01	CB1 Redistribution Assay	U2OS	•	•	•
061_01	CB2 Redistribution Assay	U2OS	•		•
097_02	GLP1R Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
8404301	Cellomics PKA Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401501	Cellomics Phospho-CREB HCS Reagent Kit	Antibody- and dye-based reagent kit			
8407101	Cellomics Phospho-GSK-3 Detection 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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