

Thermo Scientific MOR1 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.

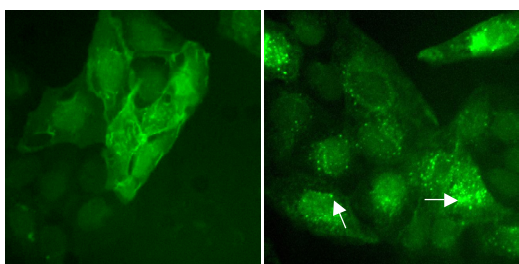


Figure 1. Internalization of MOR1-EGFP stimulated with DAMGO. Cells were treated with 10 μ M DAMGO (right panel) or untreated (DMSO control, left panel). Arrows indicate DAMGO-induced MOR1 internalization detected by the image analysis algorithm.

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Opiate drugs such as morphine modulate their activity through opioid receptors and are well known for their ability to produce potent analgesia. However, the clinical use of the opioids is limited by serious side effects such as respiratory depression, constipation, development of tolerance, and physical dependence and addiction liabilities. Three classes of opioid receptors have been pharmacologically characterized; namely the mu (μ), delta (δ), and kappa (κ) opioid receptors, where morphine primarily exerts its effect through binding of mu (μ)-opioid receptors (MORs).

Opioid receptors are G protein-coupled receptors (GPCRs) that are activated both by endogenous opioid peptides and by clinically important alkaloid analgesic drugs such as morphine. Alkaloid- or peptide-mediated MOR activation results in receptor phosphorylation by G protein coupled receptor kinase (GRK) followed by recruitment of arrestin to the receptor.

Interaction of arrestins with GPCRs results in an uncoupling of G-protein signaling from receptors (receptor desensitization) and recruitment of the endocytic machinery leading to receptor internalisation [1]. Morphine does not induce endocytosis of the activated receptor, whereas agonists such as DAMGO, etorphine, methadone, and fentanyl mediate internalization of MOR. It is the inability of morphine to promote efficient receptor internalization that has led to suggestions that morphine is particularly prone to promoting MOR desensitization and tolerance [2-5].

Features

- Designed to assay compounds for their ability to modulate internalization of MOR1
- 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

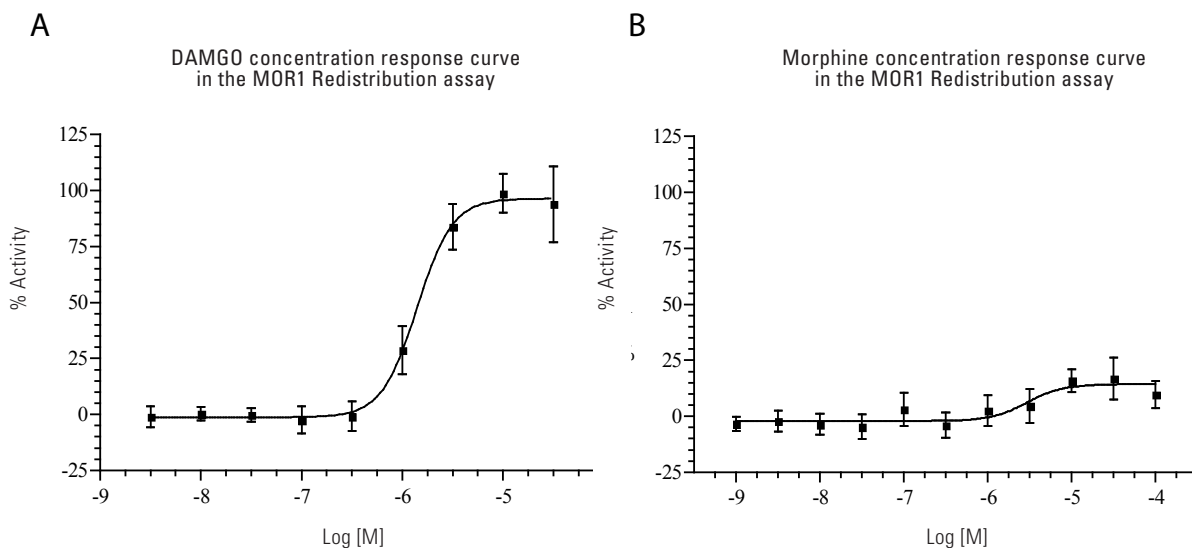


Figure 2. Concentration response curves in the MOR1 assay: A) DAMGO concentration response in the MOR1 assay (n = 12). The EC_{50} is ~1.4 μ M. Concentration response was measured in 9 point half log dilution series. Cells were treated with DAMGO for 30 min. Cells were then fixed and MOR1 internalization was measured using the Cellomics ArrayScan V^{Hi} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (10 μ M DAMGO) and negative control (0.25% DMSO). **B)** Morphine concentration response in the MOR1 assay (n=8). Concentration response was measured in 9 point half log dilution series. Cells were treated with morphine for 30 min. Cells were then fixed and MOR1 internalization was measured using the IN Cell Analyzer 3000 (GE Healthcare). % activity was calculated relative to the positive (10 μ M DAMGO) and negative control (0.25% DMSO)

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Assay Details

Recombinant U2OS cells stably expressing human mu (μ)-opioid receptor (MOR1) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists causing internalization of MOR1. DAMGO is used as reference compound. The MOR1 assay is validated with an average $Z' = 0.60 \pm 0.14$, suitable for both profiling and screening applications.

Imaging

The translocation of MOR1-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 MOR1 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^{Hi}

This assay has been validated on the Cellomics Arrayscan V^{Hi} using a 20x 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 250 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

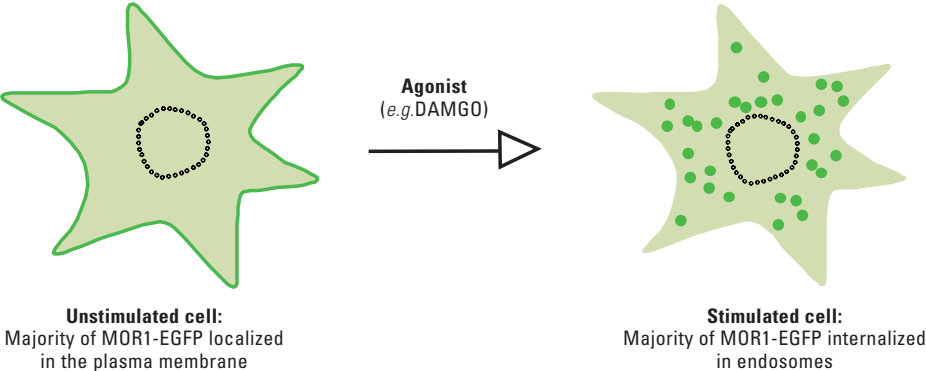


Figure 3. Illustration of the HDAC5 translocation event.

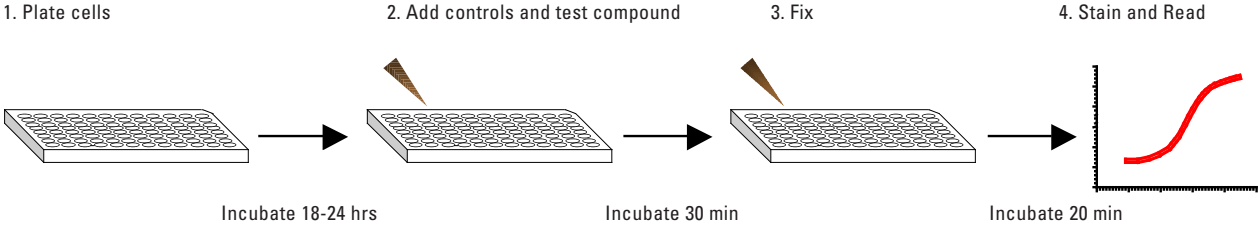


Figure 4. The MOR1 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
070_01	MOR1 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
081_01	MOR1:PKA Redistribution Assay	CHO-K1	•	•	
071_01	AT1R Redistribution Assay	U2OS	•	•	
067_01	CXCR4 Redistribution Assay	U2OS	•	•	
094_01	GRPR Redistribution Assay	U2OS	•	•	
054_01	MCH1 Redistribution Assay	U2OS	•		
039_01	S1P ₁ Redistribution Assay	U2OS	•	•	•
095_01	S1P ₃ 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			
K0100071	Cellomics ERK MAPK 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 ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

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