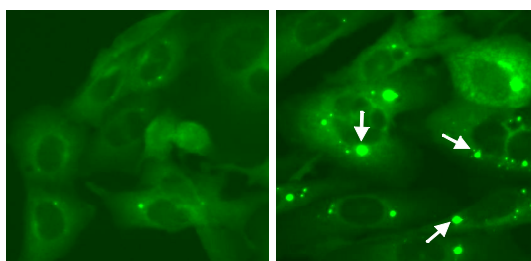


## Thermo Scientific MOR<sub>1</sub>:PKA Redistribution<sup>®</sup> Assay

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. Cytoplasmic foci formation of PKAcac-EGFP.** Cells expressing the MOR<sub>1</sub> receptor were treated with 40 μM forskolin for 15 min in the presence (right panel) or absence (left panel) of 1 μM DAMGO. Activation of the receptor inhibits the forskolin-induced dispersion of PKAcac-EGFP aggregates. Arrows indicated the cytoplasmic foci detected by the image analysis algorithm.

### Thermo Scientific MOR<sub>1</sub>:PKA Redistribution Assay

Opiate drugs modulate their activity through opioid receptors and are known for their ability to produce potent analgesia. Three classes of opioid receptors have been pharmacologically characterized; the mu (μ), delta (δ), and kappa (κ) opioid receptors. The opiate morphine works primarily by binding to the mu (μ)-opioid receptors (MORs), which are G protein coupled receptors (GPCR) coupling to G<sub>i</sub>. MOR activation results in receptor phosphorylation by GPCR kinase (GRK) followed by recruitment of arrestin to the receptor. When arrestins bind to GPCRs, the G-protein signaling is blocked and the endocytic machinery is recruited which leads to receptor internalization [1].

Morphine does not induce endocytosis of the activated MOR, whereas other agonists such as DAMGO do. In this assay, the MOR<sub>1</sub> receptor is stably transfected into a GPCR Reporter Assay cell line for G<sub>s</sub>/G<sub>i</sub>-coupled receptors. This assay system measures translocation of protein kinase A (PKA) in response to changes in the cytoplasmic cAMP concentration. Binding of a MOR agonist to the receptor causes activation

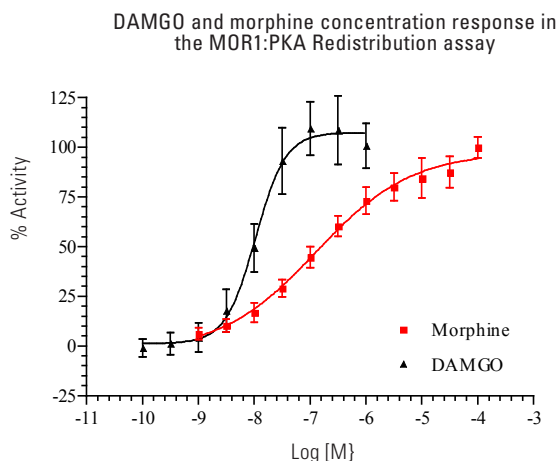
of the G<sub>i</sub> complex at the intracellular face of the receptor. This leads to inhibition of adenylate cyclase, which catalyzes the formation of cAMP from ATP. In cells stimulated with forskolin, which induces high levels of cAMP, PKAcac-GFP is found in a diffuse localization in the cytoplasm. MOR<sub>1</sub> activation leads to inhibition of adenylate cyclase and thereby less cAMP. This, in turn, leads to aggregation of PKAcac-GFP in cytoplasmic foci [2-4].

### Features

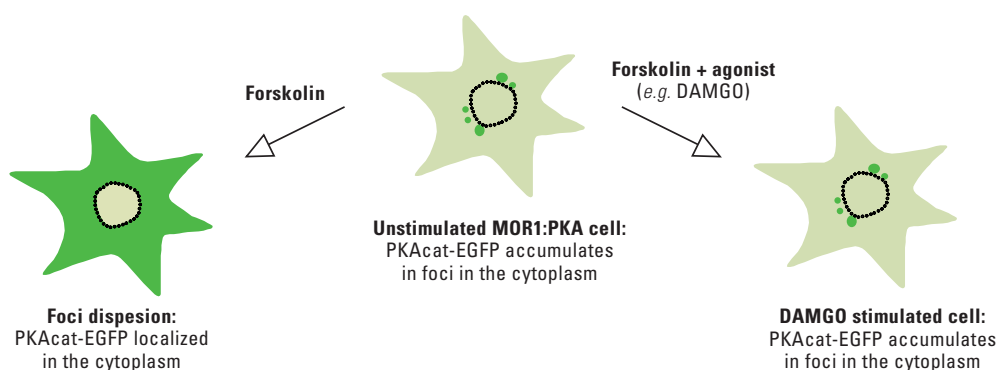
- Designed to assay compounds for their ability to inhibit a cAMP response through the MOR<sub>1</sub> receptor
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

#### 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. DAMGO and morphine concentration response in the MOR1:PKA assay.** Cells were treated with test compound in the presence of forskolin for 15 min. Cells were then fixed and cytoplasmic spot formation was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (1  $\mu$ M DAMGO) and negative control (0.25% DMSO). The EC<sub>50</sub> of DAMGO is ~10 nM (n = 16), while the EC<sub>50</sub> of morphine is ~100 nM (n = 8).



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

## Thermo Scientific MOR1:PKA Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant CHO-K1 cells stably expressing the  $\mu$  opioid receptor (MOR1) and the catalytic domain of human Protein Kinase A (PKAcat) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists causing formation of PKAcat-GFP aggregates. DAMGO is used as reference compound. The MOR1:PKA assay is validated with an average  $Z' = 0.46 \pm 0.06$ , suitable for both screening and profiling applications.

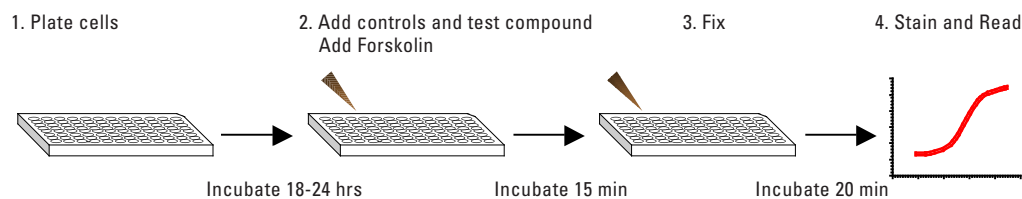
### Imaging

The translocation of PKAcat-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 10x objective or higher magnification.

The primary output in the MOR1:PKA 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>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 SpotDetectorV3 BioApplication. The output parameter used was SpotTotalIntenPerObject. 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 SpotDetectorV2, CompartmentalAnalysisV2, and ColocalizationV3.



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

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
081_01	MOR1:PKA Redistribution Assay	CHO-K1	•	•	

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
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
046_01	$\beta$ 2-AR:PKA Redistribution Assay	CHO-K1	•	•	
048_01	NK1:NFATc1 Redistribution Assay	U2OS	•	•	
067_01	S1P1:PKA Redistribution Assay	CHO-K1	•	•	
088_01	M1:NFATc1 Redistribution Assay	U2OS	•	•	
072_01	M2:PKA Redistribution Assay	CHO-K1	•	•	
073_01	M3:NFATc1 Redistribution Assay	U2OS	•	•	
078_01	AT1:NFATc1 Redistribution Assay	U2OS	•	•	
079_01	MCH1:NFATc1 Redistribution Assay	U2OS	•	•	
047_01	GlucagonR:PKA Redistribution Assay	CHO-K1	•	•	
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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

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