

Thermo Scientific M3 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.

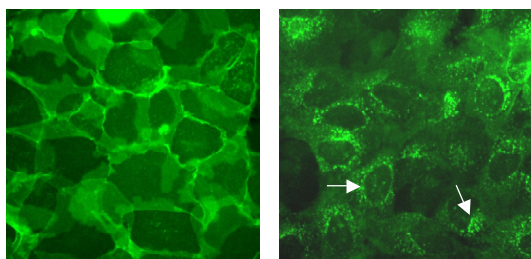


Figure 1. Internalization of M3-EGFP stimulated with carbachol. Cells were treated with 300 μ M carbachol for 1 hr (right panel) or untreated (DMSO control, left panel). Arrows indicate the M3-EGFP internalization that is detected by the image analysis algorithm.

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Muscarinic acetylcholine receptors mediate their effect by exerting metabotropic actions of acetylcholine in the nervous system. The receptors belong to the G-protein-coupled receptor (GPCR) superfamily that transduces signals through multiple intracellular signaling cascades to regulate a wide variety of physiological responses. At present, five receptor subtypes have been identified, where the odd-numbered receptors (M1, M3, M5) preferentially couple to $G\alpha_q/11$ and activate phospholipase C, thereby initiating the phosphatidylinositol trisphosphate (PIP3) cascade leading to intracellular Ca^{2+} mobilization and activation of protein kinase C. The even-numbered receptors M2 and M4 couple to $G\alpha_i/o$ and inhibit adenylyl cyclase activity.

Drugs targeting muscarinic acetylcholine receptors are currently used in the treatment of chronic obstructive pulmonary disorder and overactive bladder, but these receptors also represent potential therapeutic targets

for cognitive disorders such as Alzheimer's disease and schizophrenia. Selective M1 agonists or mixed M1 agonists/M2 antagonists may provide an approach to the treatment of cognitive disorders, while M3 antagonism or mixed M2/M3 antagonists are approved for the treatment of contractility disorders including overactive bladder and chronic obstructive pulmonary disease [1-3].

Features

- Designed to assay compounds for their ability to modulate Muscarinic acetylcholine receptor 3 (M3) internalization
- 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

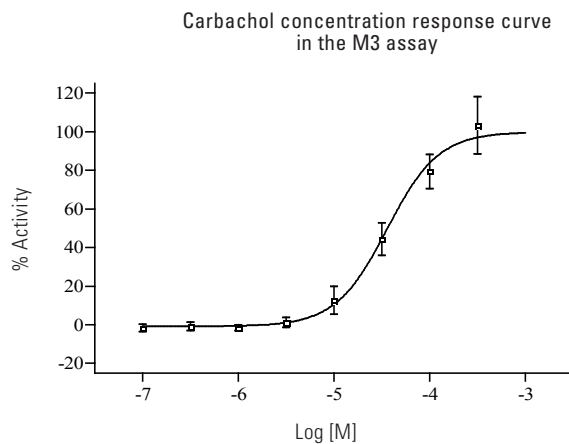


Figure 2. Carbachol concentration response in the M3 assay. Concentration response was measured in 8 point half log dilution series ($n = 16$). The EC_{50} of carbachol is $\sim 40 \mu\text{M}$. Cells were incubated with carbachol for 1 hr. Cells were then fixed and internalization was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive ($300 \mu\text{M}$ carbachol) and negative control (0.25% DMSO).

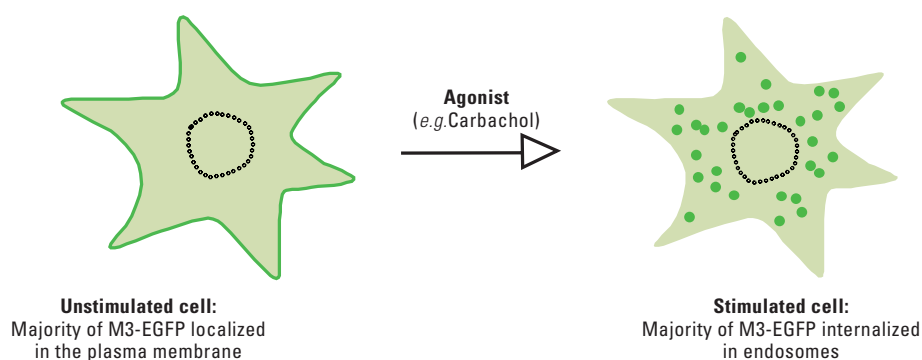


Figure 3. Illustration of the M3 translocation event.

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Assay Details

Recombinant U2OS cells stably expressing human Muscarinic acetylcholine receptor 3 (M3) fused to the N-terminus of enhanced green fluorescent protein (EGFP). Carbachol is used as reference compound and ligands/compounds are assayed for their ability to induce M3 internalization detected by a spot detecting image analysis algorithm. The M3 assay is validated with an average $Z' = 0.64 \pm 0.09$, suitable for both screening and profiling applications.

Imaging

The translocation of M3-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 M3 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^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 10x objective (0.63X coupler), High Resolution images, 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 1100 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

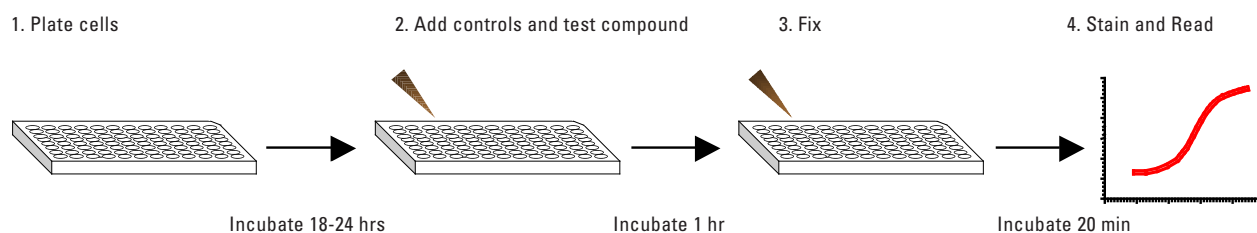


Figure 4. The M3 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
076_01	M3 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	S1P ₁ Redistribution Assay	U2OS	•	•	•
095_01	S1P ₃ Redistribution Assay	U2OS	•		•
075_01	M2 Redistribution Assay	U2OS	•	•	
086_01	M1 Redistribution Assay	U2OS	•		
057_01	MC4 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	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – 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	•	•	
K0100111	Cellomics NFAT-1 Activation 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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