

## Thermo Scientific TULP3 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.

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The Tubby protein family includes five members, namely the human homolog of murine Tubby, named TUB, and the Tubby-like proteins (TULPs) 1-4. All Tubby proteins are expressed in the retina, but TUB and TULP3 are also widely distributed in the central nervous system (CNS). TUB is implicated in obesity whereas TULP-3 is required for neural development. Tubby proteins are suggested to function in transcription and signaling stimulated by G-protein activation. Tubby proteins bind phosphatidylinositol and localize to the plasma membrane. Activation of G protein-coupled receptors (GPCRs) and subsequent signaling via the Gq subclass of G $\alpha$  proteins releases Tubby proteins from the plasma membrane by phospholipase C-mediated

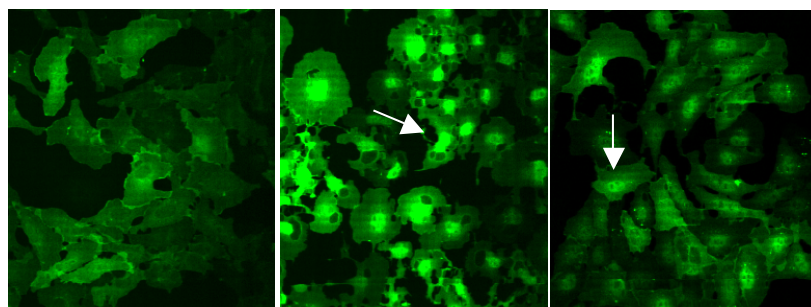
hydrolysis of PtdIns(4,5)P<sub>2</sub>. Following, the release from the membrane, Tubby proteins translocate to the nucleus [1-5].

### Features

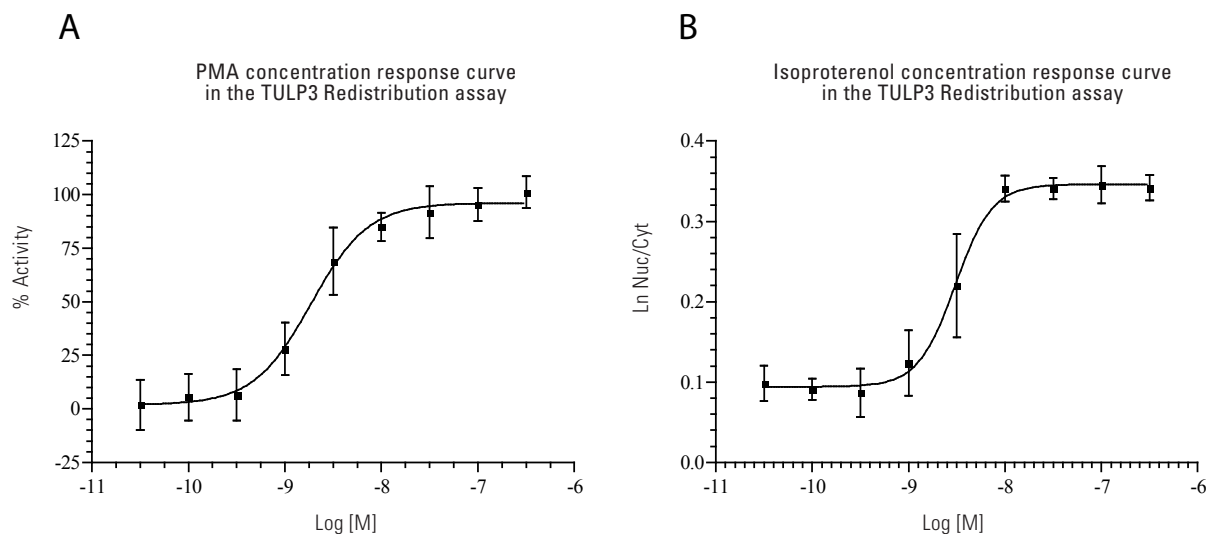
- Designed to assay compounds for their ability to modulate nuclear translocation of TULP3
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

### 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 1. Nuclear translocation of EGFP-TULP3.** Cells were untreated (DMSO control, left panel), treated with 50 nM PMA (center panel), or treated with 100 nM isoproterenol for 2 hr (right panel). Arrows indicate the nuclear localization of EGFP-TULP3 that is detected by the image analysis algorithm.



**Figure 2. Concentration response curves in the TULP3 assay: A)** PMA concentration response. The  $EC_{50}$  is approximately 2 nM. Concentration response was measured in 9 point half log dilution series ( $n = 16$ ). Cells were treated with PMA for 2 hr. Cells were then fixed and nuclear translocation was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (50 nM PMA) and negative control (0.25% DMSO). **B)** Isoproterenol concentration response. The  $EC_{50}$  is approximately 3 nM. Concentration response was measured in 9 point half log dilution series ( $n = 4$ ). Cells were analyzed using the IN Cell Analyzer 3000 (GE Healthcare).

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### Assay Details

Recombinant U2OS cells stably expressing human TULP3 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay monitors translocation of EGFP-TULP3 to the nucleus as a measure for TULP3 activation. Phorbol-12-myristate-13-acetate (PMA), an activator of protein kinase C, is used as reference compound. G-protein activity induced by the adrenoceptor agonist isoproterenol also induces TULP3 translocation. The TULP3 assay is validated with an average  $Z' = 0.43 \pm 0.1$ , suitable for both screening and profiling applications.

### Imaging

The translocation of EGFP-TULP3 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 TULP3 Redistribution assay is the translocation from cytoplasm to nucleus of EGFP-TULP3. The data analysis should therefore report an output relating to the GFP fluorescence intensities in the nucleus and 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 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 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

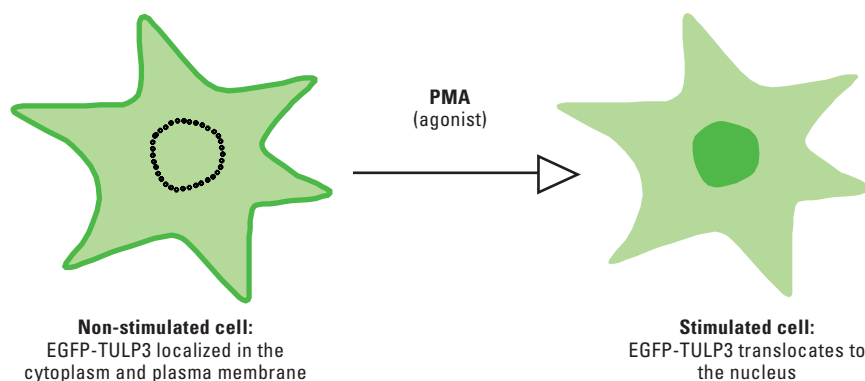


Figure 3. Illustration of the TULP3 translocation event.

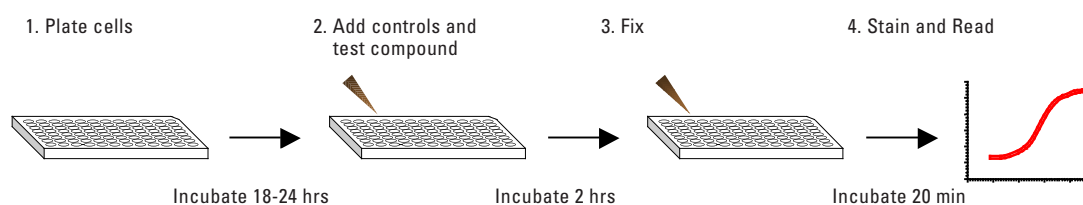


Figure 4. The TULP3 Redistribution assay is very easy and fast to perform.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
059_01	TULP3 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
096_01	MARCKS Redistribution Assay	U2OS	•	•	
098_01	PKC $\beta$ Redistribution Assay	U2OS	•		
099_01	PKC $\epsilon$ Redistribution Assay	U2OS	•	•	
K0900011	Cellomics PKC $\alpha$ Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100111	Cellomics NFAT-1 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0700011	Cellomics Neurite Outgrowth 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	CelliWoRx	Economical high content reader			

### References

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4. Santagata S et al., *Science*, 292, 2041-2050, 2001.
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