Product Specifications

Thermo Scientific ATF6 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.

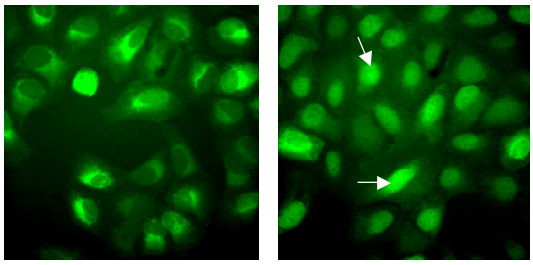

Figure 1. Translocation of EGFP-ATF6 in response to tunicamycin. Cells were treated with 1 µM tunicamycin for 5 hr (right panel) or untreated (DMSO control, left panel). Arrows indicate nuclear accumulation of ATF6 detected by the image analysis algorithm.

Thermo Scientific ATF6 Redistribution Assay

Activator of transcription 6 (ATF6) is a key transcription factor that regulates the so-called unfolded protein response (UPR). UPR is a tightly regulated process that takes place in response to physiological stress conditions resulting in aberrant protein folding in the endoplasmic reticulum (ER). During UPR, ATF6 translocates from the ER to the Golgi complex. In the Golgi ATF6 is processed into its N-terminal 50 kDa active form by a sequential process termed regulated intramembrane proteolysis (Rip) involving the Golgi site-1 and site-2 proteases (S1P and S2P). Subsequently, ATF6 translocates into the nucleus where it activates transcription of ER stress-response genes (ERSRGs) encoding molecular chaperones and enzymes ensuring proper protein folding [1-4].

Under normal conditions the majority of proteins synthesized in the ER undergo glycosylation which plays a pivotal role in protein folding, sorting, and transport. Blockage of protein glycosylation resulting in accumulation of misfolded proteins in the ER, followed by onset of the UPR, is induced by various compounds such as the antibiotic tunicamycin.

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of ATF6
- 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

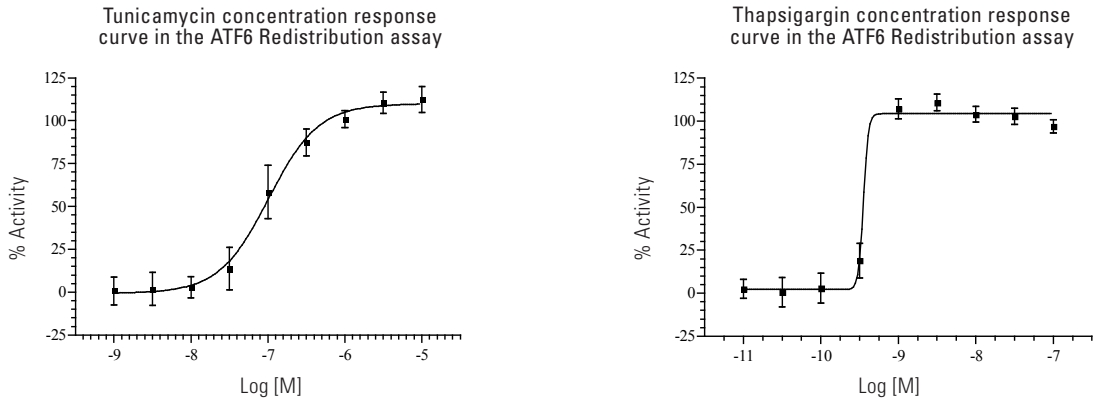

Figure 2. Tunicamycin and thapsigargin concentration response curve in the ATF6 Redistribution assay. Concentration response was measured in 9 point half log dilution series of tunicamycin (n = 16) or thapsigargin (n=8). Cells were then fixed and the ER to nucleus translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the RedistributionV3 BioApplication. % activity was calculated relative to the positive (1 µM tunicamycin) and negative control (0.25% DMSO). The EC_{50} values of tunicamycin and thapsigargin in the assay are approximately 100 nM and 0.4 nM, respectively.

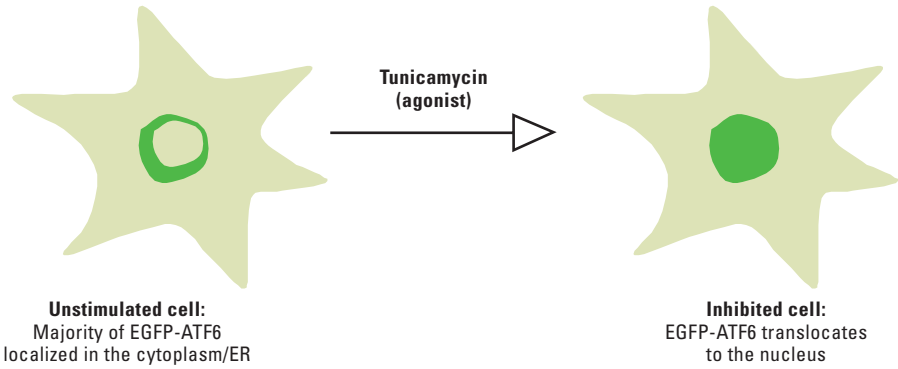

Figure 3. Illustration of the ATF6 translocation event.

Thermo Scientific ATF6 Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human ATF6 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for inducers of ER stress by monitoring the translocation of ATF6 to the nucleus. Tunicamycin is used as reference compound in the assay. The ATF6 assay is validated with an average Z' = 0.58 ± 0.09, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-ATF6 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 ATF6 Redistribution assay is the translocation of EGFP-ATF6 from the cytoplasm to the nucleus. 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^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} 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.

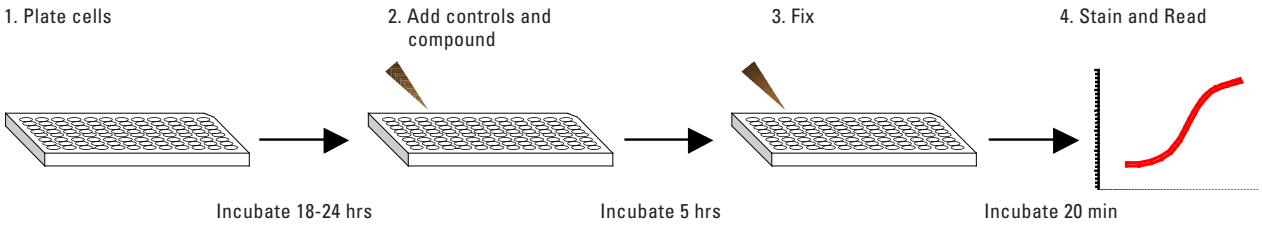

Figure 4. The ATF6 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
084_01	ATF6 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
077_01	PXR Redistribution Assay	U2OS	•	•	•
042_01	Rad51 Redistribution Assay	SW480	•	•	•
8403901	Cellomics CHOP/GADD153 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^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. Haze K. et al. *Mol Biol Cell.*, 10, 3787-3799, 1999
2. Ye J. et al. *Mol Cell.*, 6, 1355-64, 2000
3. Kaufman RJ., *J Clin Invest* 110, 1389–1398, 2002
4. Hong M. et al., *J Biol Chem*, 279, 11354–11363, 2004

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