

Thermo Scientific TORC2 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.

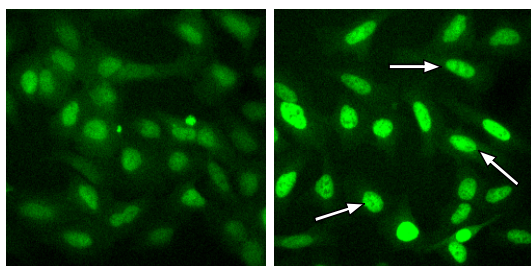


Figure 1. Nuclear translocation of EGFP-TORC2. Cells were treated with 10 μ M forskolin for 1 hr (right panel) or untreated (DMSO control, left panel). Arrows indicate the nuclear localization of EGFP-TORC2 that is detected by the image analysis algorithm.

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The TORC proteins (transducers of regulated CREB activity, TORC1, TORC2, TORC3) are regulators of the gluconeogenic program in response to hormonal and intracellular signals. TORCs are regulators of CREB activity and translocate to the nucleus after an increase in the level of intracellular cAMP or calcium. In fact, TORC proteins can be viewed as integrators of cAMP and calcium signals [1]. TORC2 shuttles between the nucleus and the cytoplasm by means of an NLS in aa 56-144, and two NES's between aa 145-320 [2]. TORC2 is dephosphorylated at Ser171 and presumable Ser369 under conditions of high levels of cAMP, leading to nuclear translocation. Nuclear TORC2 interacts with the DNA binding domain of CREB and thereby enhances gene expression of CREB target genes such as gluconeogenic genes. High levels of cAMP can be induced by glucagon, fasting treatment, or forskolin.

The effect of glucagon and/or fasting on TORC2 can be attenuated by expression of the salt-induced kinases (SIK1 or SIK2) or by activation of the AMPK pathway, e.g. by exposure to the AMP analog AICAR [3]. Compounds that induce or inhibit nuclear translocation of TORC2 are of interest as neuroprotective/memory enhancing agents or for treatment of type II diabetes, respectively.

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of TORC2
- 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

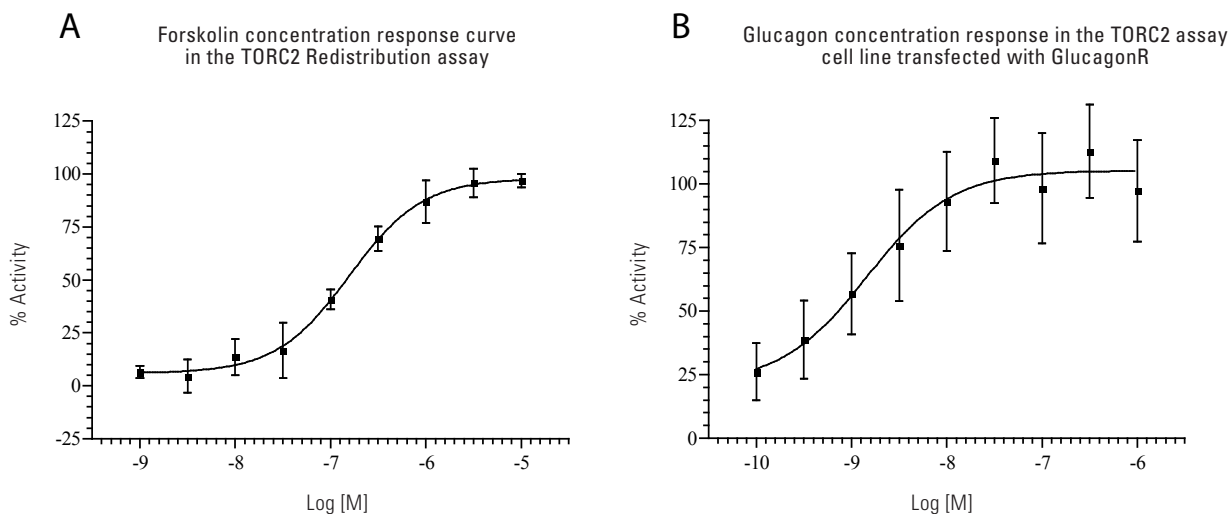


Figure 2. Concentration response curves in the TORC2 assay: A) Forskolin concentration response. The EC_{50} is approximately 160 nM. Concentration response was measured in 9 point half log dilution series ($n = 8$). Cells were treated with forskolin for 1 hr. Cells were then fixed and nuclear translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (10 μ M forskolin) and negative control (0.25% DMSO). **B)** Glucagon concentration response in the TORC2 assay cell line that was stably transfected with the glucagon receptor. Concentration response was measured in 9 point half log dilution series ($n = 8$). The EC_{50} of glucagon is approximately 2 μ M. Cells analyzed as described in A).

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Assay Details

Recombinant U2OS cells stably expressing human TORC2 fused to the C-terminus of enhanced green fluorescent protein (EGFP). In this assay compounds are tested for their ability to modulate nuclear translocation of TORC2. Forskolin is used as reference compound for induction of cAMP. The assay is also responsive to glucagon when the glucagon receptor is transfected into the assay cell line. The TORC2 assay is validated with an average $Z' = 0.62 \pm 0.07$, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-TORC2 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 TORC2 Redistribution assay is the translocation from cytoplasm to nucleus of EGFP-TORC2. 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 100 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

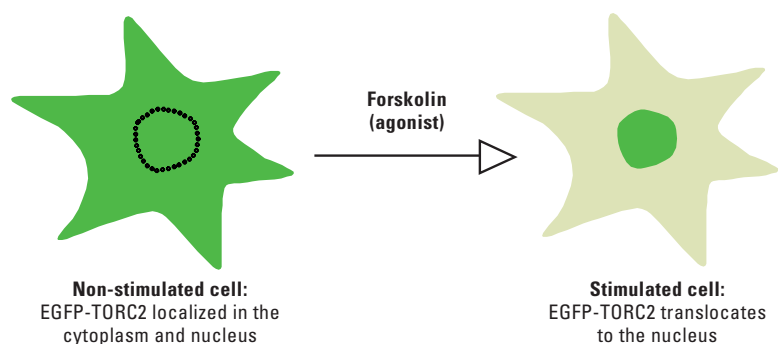


Figure 3. Illustration of the TORC2 translocation event.

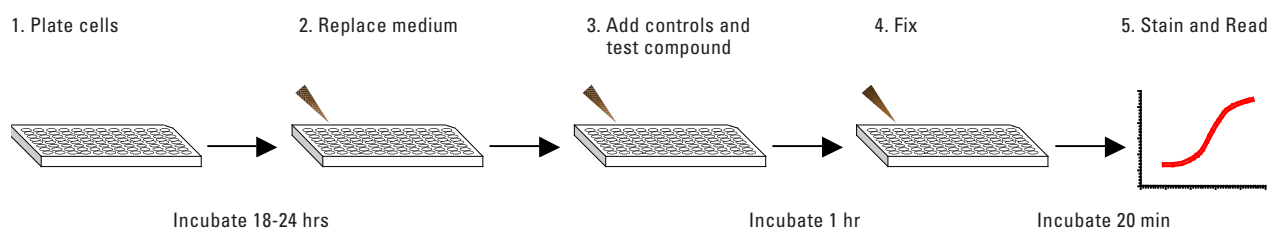


Figure 4. The TORC2 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
065_01	TORC2 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
023_01	GLUT4 Redistribution Assay	CHO	•	•	
089_01	GLUT1 Redistribution Assay	CHO	•		
097_01	GLP1R Redistribution Assay	U2OS	•	•	
8405201	Cellomics Phospho-S6 Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8405301	Cellomics Phospho-4E-BP1 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. Bittinger et al. *Curr. Biol.* 2004; 14: 2156-61
2. Screaton et al. *Cell* 2004; 119: 61-74
3. Katoh et al. *Eur. J. Biochem.* 2004; 271: 4307-19.

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