Product Specifications

Thermo Scientific MK2EE 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.

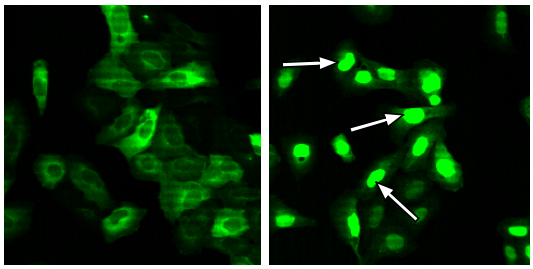

Figure 1. Translocation of EGFP-MK2EE in response to Ratjadone A. Cells were treated with (right panel) or without (left panel) 50 nM Ratjadone A. Arrows indicate Ratjadone-mediated nuclear translocation detected by the image analysis algorithm.

Thermo Scientific MK2EE Redistribution Assay

The MK2EE Redistribution assay is designed as a control assay for the MK2 Redistribution assay to distinguish inhibitors acting upstream of MK2 from inhibitors specifically modulating MK2 translocation. In addition, the MK2EE assay can be used to identify compounds inhibiting nuclear export of MK2, irrespective of its activation state. In the MK2 assay, MK2 translocates from the nucleus to the cytoplasm upon p38 MAPK pathway stimulation (*e.g.* interleukin-1), but the two mutations (T222E and T334E) in the MK2EE assay construct result in constitutive activity and hence cytoplasmic localization of MK2EE [1-4].

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of MK2EE
- 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

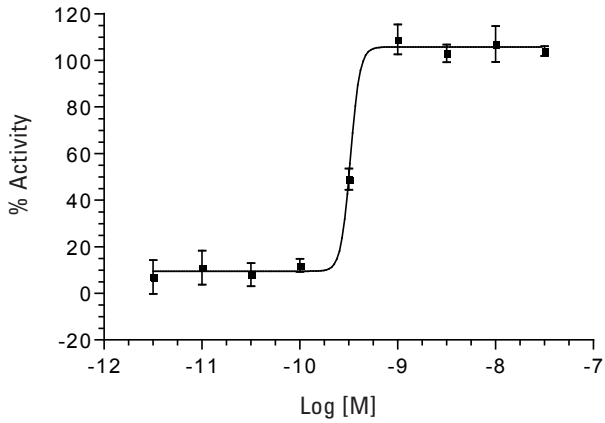

Figure 2. Concentration-response curve of Ratjadone A in the MK2EE Redistribution assay (n = 8). Concentration response was measured in 9 point half log dilution series of Ratjadone A. Cells were incubated with Ratjadone A for 60 min. Cells were then fixed and the nucleus to cytoplasm translocation was measured using image analysis. % activity was calculated relative to the positive (50 nM Ratjadone A) and negative control (0.25% DMSO).

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

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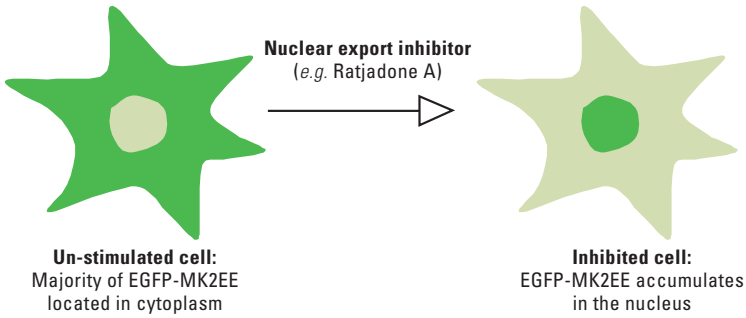

Figure 3. Illustration of the MK2EE translocation event.

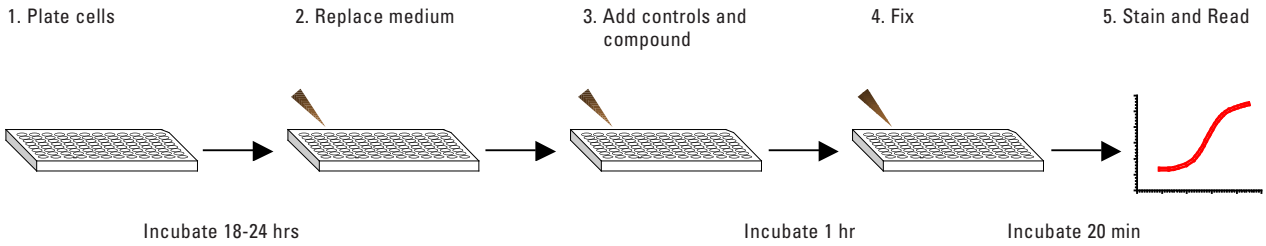

Figure 4. The MK2EE Redistribution assay is very easy and fast to perform.

Thermo Scientific MK2EE Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human MAPKAPK2 (MK2) isoform 2 fused to the C-terminus of enhanced green fluorescent protein (EGFP). In the MK2EE assay, compounds are assayed for their ability to inhibit the cytoplasmic localization of MK2EE, and the specific inhibitor of Crm1-mediated nuclear export Ratjadone A is used as a reference compound [5]. The activity of Ratjadone A in the assay is equivalent to the activity obtained with the natural export inhibitor Leptomycin B. Compounds which inhibit cytoplasmic localization of MK2EE either directly interfere with MK2 translocation (and not upstream activities), or they act as general nuclear export inhibitors. The assay can be used alone to assay for compounds inhibiting nuclear export, or it can be used in combination with the MK2 Redistribution assay to distinguish inhibitors acting upstream of MK2 from MK2-specific inhibitors, which act independently of MK2 activity. If the assay is used in combination with the MK2 Redistribution assay, Ratjadone A should be used as reference compound in both assays. The MK2EE assay is validated with an average Z′ = 0.62 ± 0.04, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-MK2EE 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 MK2EE Redistribution assay is the translocation of EGFP-MK2EE 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}

We recommend running the assay 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, using the output 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 is set to 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2 and ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
038_01	MK2EE 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
037_01	MK2 Redistribution Assay	U2OS	•	•	
062_01	REV nuclear export Redistribution Assay	U2OS	•	•	
K0100031	Cellomics Phospho-c-Jun Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100011	Cellomics NFkB Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8406001	Cellomics Hsp27 and Phospho-Hsp27 Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100041	Cellomics p38 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

1. Engel K et al., *J Biol Chem* 270, 27213-27221, 1995.
2. Ben-Levy R et al., *EMBO J* 14, 5920-30, 1995.
3. Engel K et al., *EMBO J* 17, 3363-3371, 1998.
4. Ben-Levy R et al., *Cur Biol* 8, 1049-1057, 1998.
5. Kalesse M et al., *Chembiochem* 2, 709-714, 2001.

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