

Thermo Scientific ERF1 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.

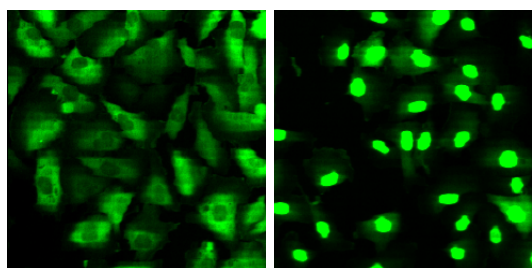


Figure 1. Translocation of EGFP-ERF1 in response to U0126. Cells were treated without (DMSO control, left panel) or with (right panel) 10 μ M U0126. The U0126-mediated nuclear translocation is detected by the image analysis algorithm.

Thermo Scientific ERF1 Redistribution Assay

The Ets2 Repressor Factor (ERF/ERF1) is a transcriptional repressor and member of the Ets family of transcription factors. ERF1 antagonizes the activity of other Ets family members that function as transcriptional activators. The activity of ERF1 is regulated by the Ras/MEK/Erk signaling pathway that links extracellular signals with cell proliferation and differentiation. Erk-dependent phosphorylation of ERF controls the activity of ERF1 by modulating its cellular localization. Thus, upon mitogenic stimulation ERF1 is immediately phosphorylated by Erk and exported to the cytoplasm. Conversely, upon serum withdrawal, ERF1 is rapidly dephosphorylated and imported into the nucleus. ERF1 nuclear export is a CRM-1 dependent process and is blocked by general inhibitors of nuclear export such as Leptomycin B and Ratjadone A [1, 2, 3]. The ERF1 Redistribution assay is designed to assay for inducers of ERF1 translocation by monitoring the translocation of an EGFP-ERF1 fusion protein from the cytoplasm to

the nucleus in the human bladder cancer cell line T24. T24 cells express constitutively active Ha-Ras resulting in a cytoplasmic localization of ERF1. Inhibition of the Ras/Erk signaling pathway by the MEK inhibitor U0126 [4-6] prevents phosphorylation and results in nuclear localization of ERF1.

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of ERF1
- 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

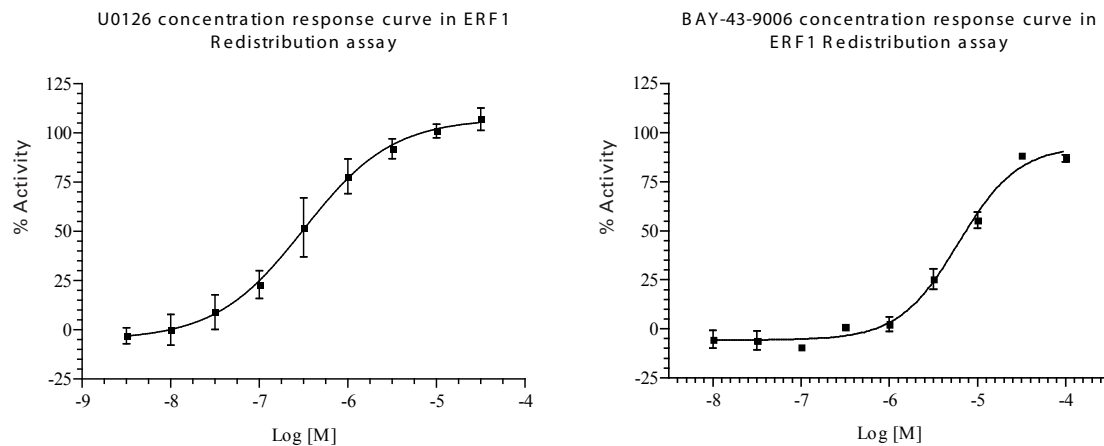


Figure 2. Concentration response curves in the ERF1 Redistribution assay. Concentration response was measured in 9 point half log dilution series. Cells were incubated with test compound for 1 hour and fixed before image analysis to detect cytoplasm to nucleus translocation. A) U0126 concentration response curve in the ERF1 Redistribution assay (n=11). The EC_{50} of U0126 is approximately 320 nM. B) BAY-43-9006 concentration response curve in the ERF1 Redistribution assay (n=3). The EC_{50} of BAY-43-9006 is approximately 6 μ M. % activity was calculated relative to the positive (10 μ M U0126) and negative control (0.25% DMSO).

Thermo Scientific ERF1 Redistribution® Assay

Assay Details

Recombinant T24 cells stably expressing human ERF1 (Ets2 repressor factor) fused to the C-terminus of enhanced green fluorescent protein (EGFP). In this assay U0126 is used as reference compound and compounds are assayed for their ability to induce nuclear accumulation of ERF1. An ERF1 Redistribution assay developed in U2OS human osteosarcoma cells is also available. Compounds causing nuclear accumulation of ERF1 may directly interfere with ERF1 import, act upstream of ERF1 by interfering with the Ras/Erk pathway, or may be general nuclear import activators/nuclear export inhibitors. Compounds that induce nuclear accumulation of ERF1 can be analyzed for general export inhibitor characteristics using the Rev1 Redistribution Assay. The ERF1 assay is validated with an average $Z' = 0.73 \pm 0.11$, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-ERF1 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 ERF1 Redistribution assay is the translocation

from cytoplasm to nucleus of EGFP-ERF1. 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[®]

This assay has been developed on the Cellomics ArrayScan V[®] 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.

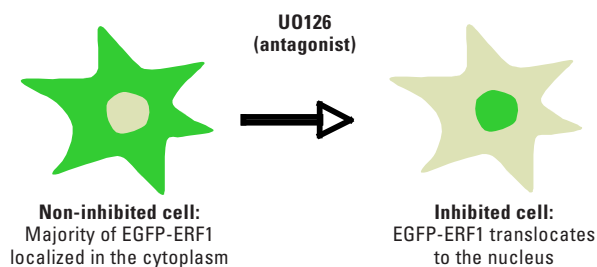


Figure 3. Illustration of the ERF1 translocation event.

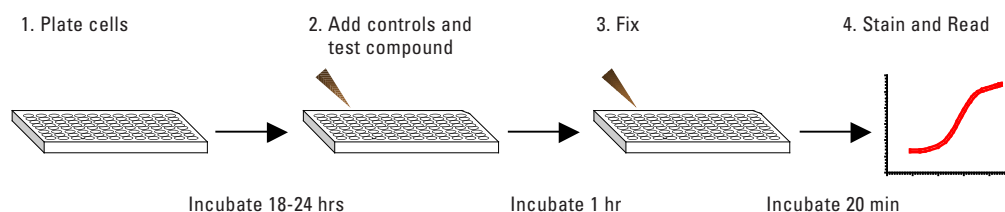


Figure 4: The ERF1 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
019_01	ERF1_T24 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
022_01	ERF_U2OS Redistribution Assay	U2OS	•	•	•
087_01	ERK2 Redistribution Assay	U2OS	•		
082_01	EGFR antagonist Redistribution Assay	U2OS	•		
049_01	Ras activation Redistribution Assay	CHO	•		
K0100071	Cellomics ERK MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100041	Cellomics p38 MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404001	Cellomics Phospho-JNK Detection Kit 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. Le Gallic L et al., *Mol Cell Biol* 19, 4121-4133, 1999.
2. Mavrothalassitis G & Ghysdael J, *Oncogene*, 19, 6524-32, 2000.
3. Le Gallic L et al., *Mol Cell Biol* 24, 1206-1218, 2004.
4. Favata MF et al., *J. Biol Chem* 273, 18623-18632, 1998.
5. Duncia JV et al., *Bioorg Med Chem Lett* 1998 8, 2839-2844, 1998.
6. DeSilva DR et al., *J Immunol* 160, 4175-4181, 1998.

