

Thermo Scientific ERK2 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.

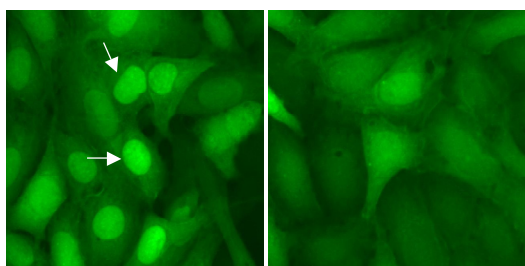


Figure 1. Inhibition of nuclear accumulation of EGFP-ERK2 by U0126. Cells were treated with EGF alone (control, left panel) or with EGF + 30 μ M U0126 for 60 min (right panel). Arrows indicate the EGF-induced nuclear localization that is inhibited by U0126 and that is detected by the image analysis algorithm.

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The ubiquitously expressed extracellular signal-regulated kinases (ERKs)/mitogen-activated protein kinases (MAPKs) function to transmit signals from growth factors and hormones to intracellular responses. Two major isoforms of ERK exist: ERK1 (p44) and ERK2 (p42). In resting cells, unphosphorylated ERK2 is anchored in the cytoplasm by its activator MAPK/ERK kinase 1 (MEK1). Upon relevant activation signals, MEK1 phosphorylates and activates ERK2, leading to its nuclear translocation. Activated ERK2 functions to regulate several target genes encoding proteins that control cellular processes such as cell proliferation, differentiation, and survival.

Inactivation of ERK2 is associated with its export from the nucleus. Nuclear export of ERK2 is also mediated by MEK1. For nuclear export, ERK2 binds to MEK1 that via association of its nuclear export sequence (NES) connects with the nuclear export receptor CRM1. This process can be inhibited by leptomycin B, which

disrupts CRM1-mediated nuclear export. Finally, export of ERK2 from the nucleus is also regulated via action of Ser/Thr phosphatases (PPs) and dual specificity phosphatases termed MAPK phosphatases (MKPs). [1-4].

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of ERK2
- 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

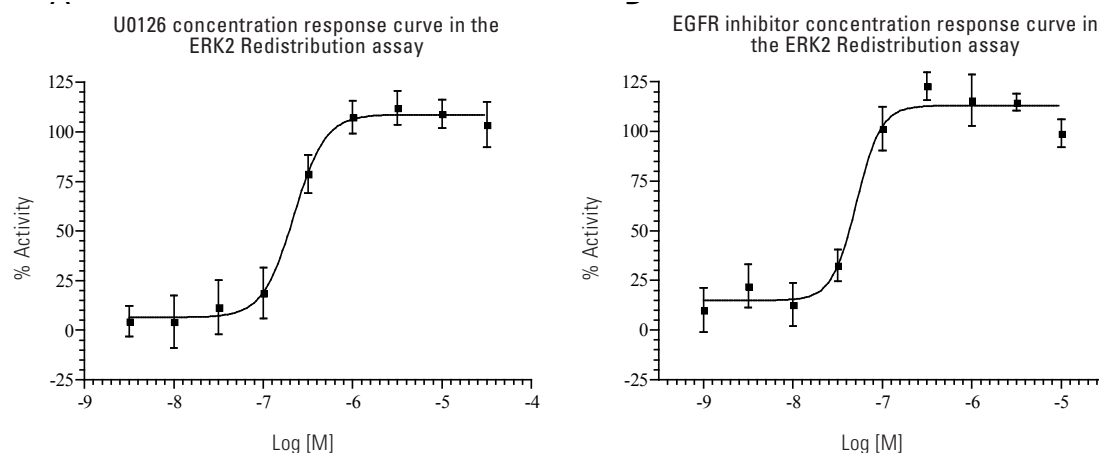


Figure 2. Concentration response curves in the ERK2 assay: A) U0126 concentration response (n = 16). The EC_{50} is approximately 0.2 μ M. **B)** EGFR inhibitor concentration response (n = 8). The EC_{50} is approximately 55 nM. Concentration response was measured in 9 point half log dilution series. Cells were incubated with test compound for 1 hr, then treated with 30 ng/ml EGF for 4 min. 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 (30 μ M U0126) and negative control (0.25% DMSO)

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Assay Details

Recombinant U2OS cells stably expressing human ERK2 fused to the N-terminus of enhanced green fluorescent protein (EGFP) and human MEK1. The assay is designed to screen for antagonists of EGF-induced translocation of ERK2 from the cytoplasm to the nucleus in the human osteosarcoma cell line U2OS. These cells also express MEK1 essential for ERK2 activity. The MEK1 inhibitor U0126 is used as a reference compound in the assay. Test compounds are assayed for their ability to inhibit nuclear accumulation of ERK2. The ERK2 assay is validated with an average $Z' = 0.53 \pm 0.13$, suitable for profiling applications.

Imaging

The translocation of EGFP-ERK2 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 ERK2 Redistribution assay is the translocation

of EGFP-ERK2 between the cytoplasm and 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 650 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

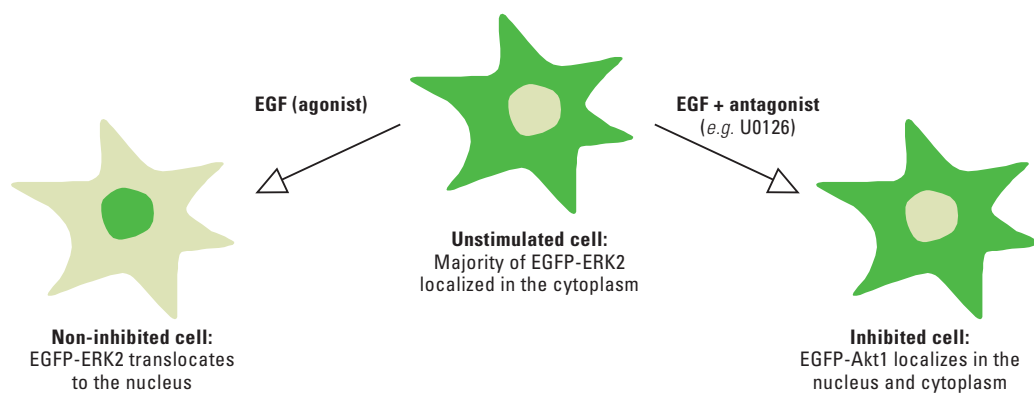


Figure 3. Illustration of the ERK2 domain translocation event.

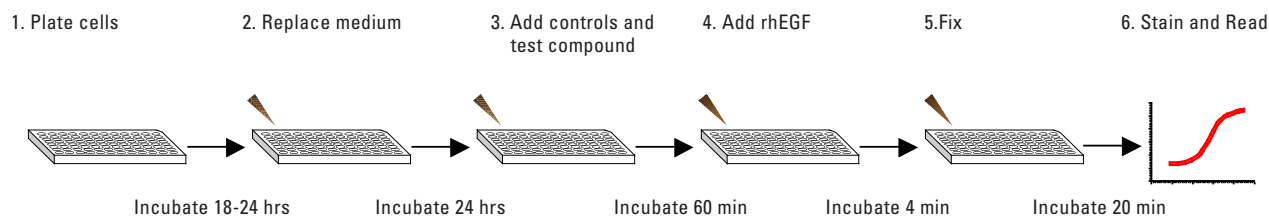


Figure 4. The ERK2 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
087_01	ERK2 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
016_01	STAT3 EGF-stimulated Redistribution Assay	MDA-MB-468	•	•	•
049_01	Ras activation Redistribution Assay	CHO	•		
019_01	ERF, MAPK pathway reporter Redistribution Assay	T24	•	•	•
022_01	ERF, MAPK pathway reporter Redistribution Assay	U2OS	•	•	•
082_01	EGFR Redistribution Assay	U2OS	•		
K0100071	Cellomics ERK 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

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- Pearson G et al., *Endocr Rev*, 22, 153–183, 2001.
- Rubinfeld H et al., *J Biol Chem*, 274, 30349–52, 1999.
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