

## Thermo Scientific EGFR Redistribution<sup>®</sup> 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.

### Thermo Scientific EGFR Redistribution Assay

Epidermal growth factor receptor (EGFR, ErbB-1) is a member of the ErbB receptor family, a subfamily of four closely related tyrosine kinases [1]. The other members of the ErbB receptor family are HER2/c-neu (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4). EGFR is a ubiquitous single-transmembrane receptor, which in its inactive state is present as monomers in the cell membrane.

Upon activation by a ligand, the receptor dimerizes with another member of ErbB receptor family, *e.g.* another EGFR molecule or HER2. The homo- and heteromer ErbB receptor dimers have different ligands. EGFR dimers and EGFR/ErbB-2 heterodimers are activated by epidermal growth factor (EGF), transformation growth factor  $\alpha$  (TGF $\alpha$ ), and amphiregulin (AR). Binding of ligand to the receptor and subsequent dimerization leads to stimulation of the intrinsic tyrosine kinase activity of EGFR and activation of multiple downstream signaling pathways,

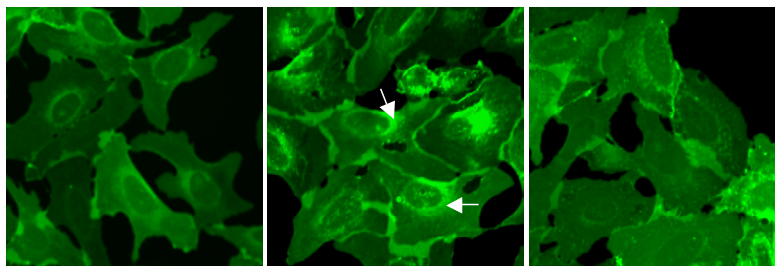
including the MAP kinase pathway, the AKT pathway, and the STAT pathway. Following activation, the EGFR is internalized through clathrin coated pits and subsequently sorted to and degraded in lysosomes. The ubiquitin ligase c-Cbl targets the EGFR to endocytosis by tagging the receptor with multiple monoubiquitin molecules important for receptor down-regulation [2-4]. The EGFR and HER2 are involved in cell proliferation and differentiation, and they are often over-expressed or amplified in cancers, making them important targets for cancer therapy [5].

### Features

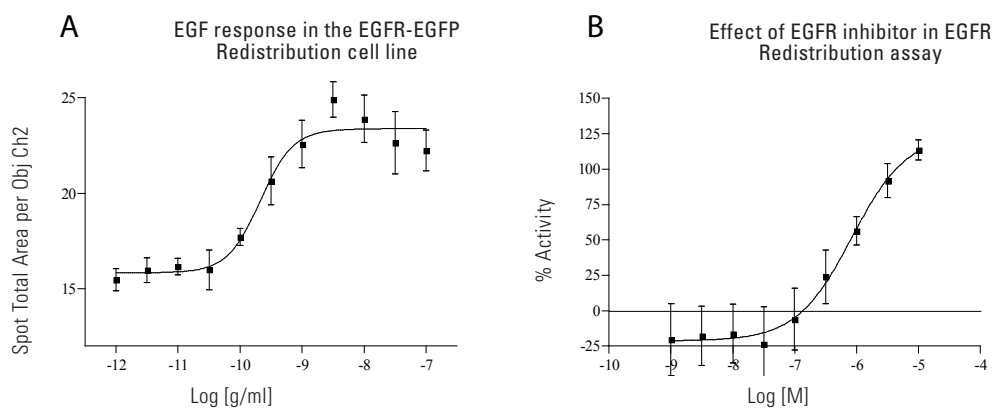
- Designed to assay compounds for their ability to modulate internalization of EGFR
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

### Highlights:

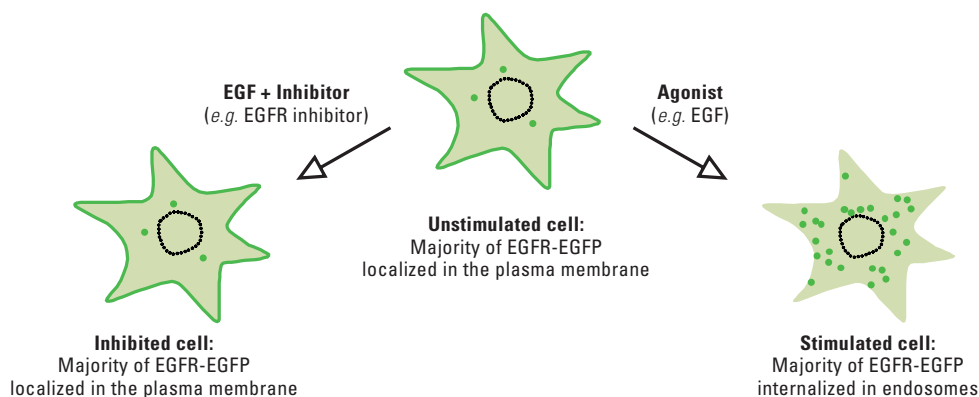
- **Biologically relevant data**  
Compounds tested in a cellular environment
- **Validated**  
Functionally tested cells provided with an optimized assay protocol
- **Easy to use**  
Just plate cells, add compounds, and image



**Figure 1. Internalization of EGFR-EGFP in response to EGF.** Cells were untreated (DMSO control, left panel), or treated with 10 ng/ml EGF for 30 min in the presence (right panel) or absence (center panel) of EGFR inhibitor. Arrows indicate the EGFR-EGFP internalization that is detected by the image analysis algorithm.



**Figure 2. A) Concentration response of EGF in the EGFR Redistribution assay (n = 8).** Cells were treated with EGF for 30 min. Cells were then fixed and receptor internalization was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the SpotDetectorV3 BioApplication. The EC<sub>50</sub> of EGF is ~0.2 ng/ml. **B) Concentration response of the EGFR inhibitor compound in the EGFR Redistribution assay (n=16).** Cells were incubated with compound for 1 hr, followed by EGF treatment for 30 min. Cells were then fixed and receptor internalization was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the SpotDetectorV3 BioApplication. The EC<sub>50</sub> of the EGFR inhibitor is ~1 μM.



**Figure 3.** Illustration of the EGFR translocation event.

## Thermo Scientific EGFR Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant U2OS cells stably expressing human epidermal growth factor receptor (EGFR) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for antagonists of EGF-induced internalization of EGFR from the plasma membrane to endosomes of human U2OS osteosarcoma cells. EGFR inhibitor [6] is used as reference compound in the assay, and compounds are assayed for their ability to inhibit internalization of the EGFR. The EGFR assay is validated with an average  $Z' = 0.37 \pm 0.2$  and is suitable for profiling applications.

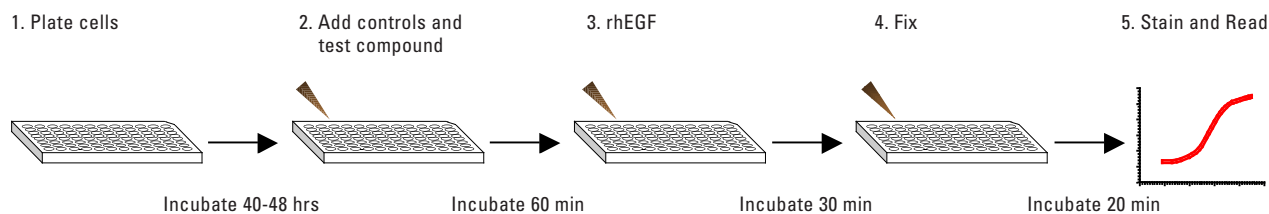
### Imaging

The translocation of EGFR-EGFP 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 EGFR Redistribution assay is the formation of spots in the cytoplasm. The data analysis should therefore report an output that corresponds to number, area, or intensity of spots in the cytoplasm.

### Imaging on Thermo Scientific Cellomics ArrayScan V<sup>TI</sup>

This assay has been validated on the Cellomics Arrayscan V<sup>TI</sup> using a 10x objective (0.63X coupler), High Resolution images, XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. The minimally acceptable number of cells used for image analysis in each well was set to 450 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.



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

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
082_01	EGFR 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	•	•	•
087_01	ERK2 Redistribution Assay	U2OS	•		
062_01	REV 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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

### References

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2. Mosesson Y et al., *J Biol Chem.*, 278, 21323–21326, 2003.
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4. Jiang X and Sorkin A. *Traffic*, 4, 529–543, 2003.
5. Johnston JB et al., *Curr Med Chem*. 13, 3483-3492, 2006.
6. Calbiochem, Cat no. 324674, *Cyclopropanecarboxylic acid-(3-(6-(3-trifluoromethyl-phenylamino)-pyrimidin-4-ylamino)-phenyl)-amide*.

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