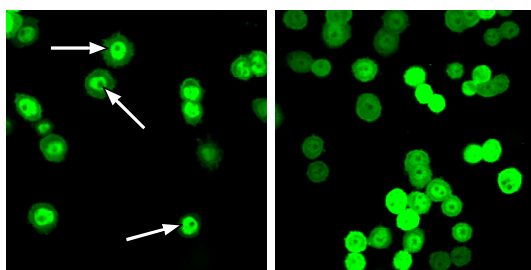


## Thermo Scientific STAT3 (hEGF) Redistribution<sup>®</sup> Assay

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.



**Figure 1. Translocation of EGFP-STAT3 stimulated with EGF in response to PD158780.** Cells were treated with 100 ng/mL EGF in the absence (DMSO control, left panel) or presence (right panel) of 1  $\mu$ M PD158780. Arrows indicate EGF-mediated nuclear translocation detected by the image analysis algorithm.

### Thermo Scientific STAT3 (hEGF) Redistribution Assay

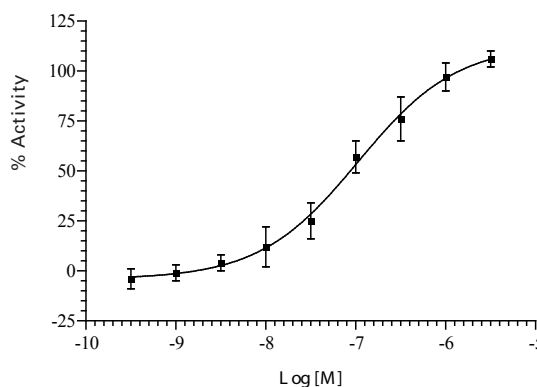
Signal transducers and activators of transcription (STAT) is a family (STAT 1, 2, 3, 4, 5a, 5b, and 6) of cytoplasmic proteins that function as secondary messengers and transcription factors. STAT proteins are activated via phosphorylation by Janus kinases (JAKs) in response to cytokine activation of cell surface receptor tyrosine kinases. Interestingly, epidermal growth factor (EGF) upon binding to the EGF receptor (EGFR), also activates STAT signalling pathways either dependently or independently of JAKs [1]. Following STAT activation the proteins dimerize and localize to the nucleus where they activate transcription of a selected set of genes.

It is known that STAT3 regulates important functions such as embryogenesis, apoptosis, skin remodelling, cell migration as well as macrophage inactivation and down-regulation of inflammatory cytokines in T-helper-cell responses [2].

#### Features

- Designed to assay compounds for their ability to modulate nuclear translocation of STAT3
- 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

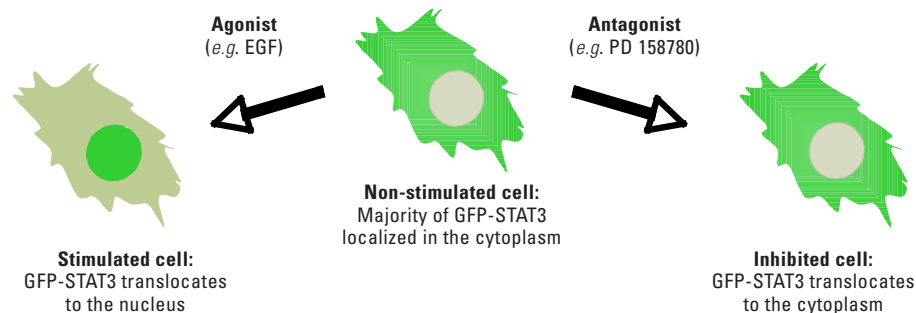
PD158780 concentration response curve  
in STAT3 (hEGF) assay



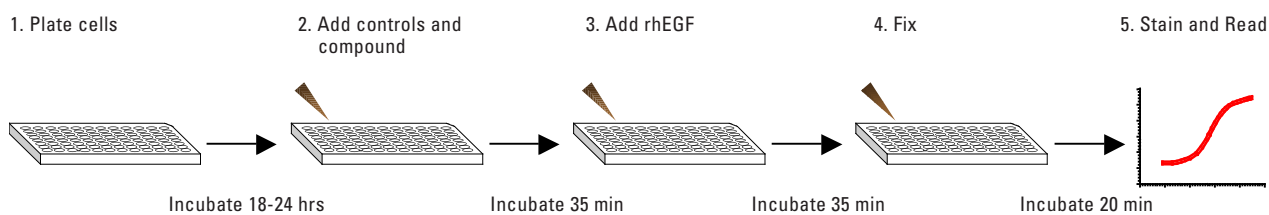
**Figure 2: PD158780 concentration response curve in the STAT3 (hEGF) Redistribution assay (n=8).** Concentration response was measured in 9 point half log dilution series of PD158780. Cells were then fixed and the nucleus to cytoplasm translocation was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the RedistributionV3 BioApplication. % activity was calculated relative to the positive (1  $\mu$ M PD158780) and negative control (0.25% DMSO). The EC<sub>50</sub> value of PD158780 is approximately 107 nM in the assay.

#### 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 3.** Illustration of the STAT3 translocation event.



**Figure 4.** The STAT3 Redistribution assay is very easy and fast to perform.

## Thermo Scientific STAT3 (hEGF) Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant MDA-MB-468 cells stably expressing human STAT3 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The STAT3 Redistribution assay is designed to assay for antagonists of STAT3 translocation by monitoring the translocation of an EGFP-STAT3 fusion protein. Human EGF is used as a reference agonist and test compounds are assayed for their ability to inhibit EGF-induced cytoplasm-to-nucleus translocation of the EGFP-STAT3 fusion protein. The EGF receptor (EGFR) inhibitor PD 158780 blocks STAT3 phosphorylation as well as its subsequent nuclear import and is used in the assay as a reference antagonist [3]. Another EGFR inhibitor, BPIQ-II, is also active in the assay [4]. Compounds inhibiting EGF-induced translocation may interfere directly with STAT3 translocation, act upstream of STAT3, or be general nuclear import inhibitors or nuclear export activators. Notably, in this assay EGF activates STAT3 in a JAK-independent manner. When run in parallel with the interleukin-6-mediated STAT3 Redistribution assay, it is possible to distinguish between inhibitors of JAK-dependent and JAK-independent STAT3 activation (see related products). The STAT3 assay is validated with an average  $Z' = 0.58 \pm 0.05$ , suitable for both screening and profiling applications.

### Imaging

The translocation of STAT3 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 20x objective or higher magnification. The primary output in the STAT3 Redistribution assay is the translocation of STAT3 between the cytoplasm and 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<sup>™</sup>

This assay has been developed on the Cellomics ArrayScan V<sup>™</sup> using a 20x 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.

**Ordering Information**

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
016_01	STAT3 Redistribution Assay (hEGF)	MDA-MB-468	•	•	•

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 Redistribution Assay (hIL-6)	U2OS	•	•	•
035_01	STAT1 Redistribution Assay	U2OS	•	•	•
K0100021	Cellomics STAT1 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100051	Cellomics STAT2 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100081	Cellomics STAT3 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100011	Cellomics NFkB Activation 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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

**References**

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3. Fry DW et al. *Biochem Pharmacol.* 54, 877-887, 1997.
4. Rewcastle GW et al. *J. Med. Chem.* 39, 918-928, 1996.

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