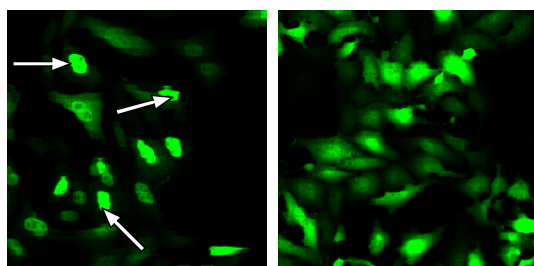


## Thermo Scientific STAT3 (hIL-6) Redistribution<sup>®</sup> Assay

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 hIL-6 in response to JAK inhibitor 1.** Cells were treated with 100 ng/mL hIL-6 in the absence (DMSO control, left panel) or presence (right panel) of 3  $\mu$ M JAK inhibitor 1. Arrows indicate IL-6-mediated nuclear translocation detected by the image analysis algorithm.

### Thermo Scientific STAT3 (hIL-6) Redistribution Assay

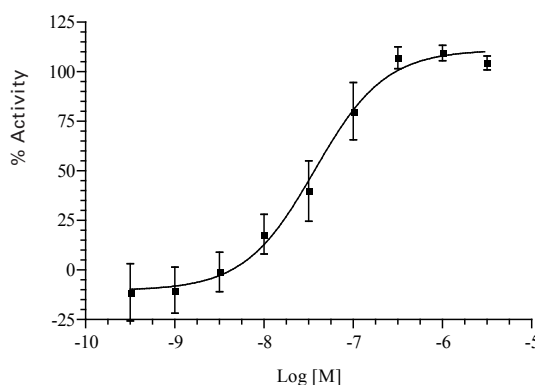
Signal transducer and activator of transcription 3 (STAT3) is involved in early embryogenesis, epithelial cell apoptosis, skin remodelling, and keratinocyte migration, as well as in macrophage inactivation and down regulation of inflammatory cytokines in T-helper-cell responses. STAT3 plays a central role in transmitting signals from the membrane to the nucleus, and binding of ligands such as the cytokine interleukin-6 (IL-6) to its receptor activates the Janus kinase (JAK)/STAT signalling pathway [1]. Inactive STAT3 is cytoplasmic, but upon activation it is rapidly recruited to activated receptors, where its association with JAK catalyzes

ligand-induced phosphorylation of STAT3. This leads to an SH2-mediated dimerization of STAT3, followed by translocation to the nucleus and activation of cytokine-responsive genes.

#### 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

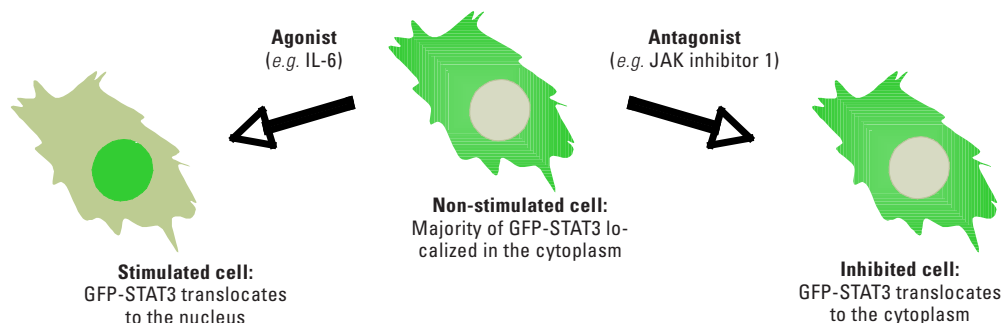
Jak Inhibitor 1 concentration response curve  
in STAT3 Redistribution assay



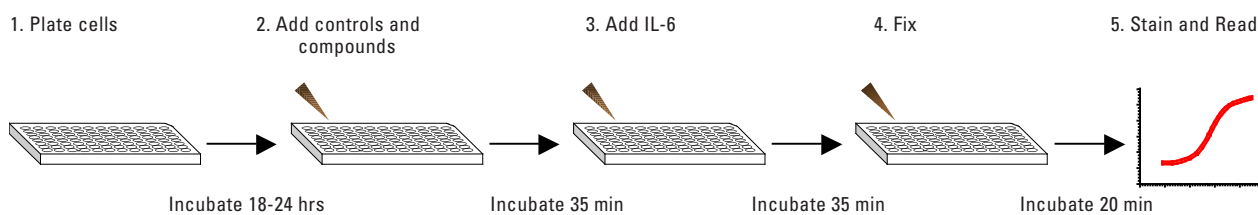
**Figure 2: JAK inhibitor 1 concentration response curve in the STAT3 (hIL-6) Redistribution assay (n=12).** Concentration response was measured in 9 point half log dilution series of Jak Inhibitor 1. Cells were then fixed and the nucleus to cytoplasm translocation was measured using the Cellomics ArrayScan V<sup>®</sup> Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (3  $\mu$ M Jak Inhibitor 1) and negative control (0.25% DMSO). The EC<sub>50</sub> of Jak Inhibitor 1 is approximately 40 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 (hIL-6) Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant U2OS cells stably expressing human STAT3 (GenBank Acc. NM\_003150) 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 a GFP-STAT3 fusion protein from the cytoplasm to the nucleus. Human interleukin-6 is used as the reference agonist compound and test compounds are assayed for their ability to inhibit interleukin-induced cytoplasm-to-nucleus translocation of STAT3. STAT3 nuclear translocation can be inhibited by JAK inhibitor 1, thereby blocking STAT3 phosphorylation and subsequent nuclear import. In this assay, JAK inhibitor 1 is used as a reference antagonist [2]. Compounds inhibiting interleukin-6-induced translocation could be interfering directly with STAT3 translocation, acting upstream of STAT3, or could be general nuclear import inhibitors/nuclear export activators. A JAK inhibitor-insensitive STAT3 Redistribution assay in MDA-MB-468 cells is also available. This assay can be used for distinguishing JAK inhibitors from compounds which directly inhibit STAT3 nuclear import when compounds are run in parallel in the IL-6-mediated and the EGF-mediated STAT3 Redistribution assay, respectively (see related products). The STAT3 assay is validated with an average  $Z' = 0.64 \pm 0.08$ , 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 10x 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 validated on the Cellomics ArrayScan V<sup>™</sup> 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.

**Ordering Information**

| PRODUCT # | DESCRIPTION                        | CELL LINE | PROFILING | SCREENING | CRYOREDI |
|-----------|------------------------------------|-----------|-----------|-----------|----------|
| 015_01    | STAT3 Redistribution Assay (hIL-6) | 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 Redistribution Assay (EGF)           | MDA-MB-468  | •         | •         | •        |
| 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                 |           |           |          |
| 8403801     | Cellomics iNOS 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**

1. Zhong Z, Wen Z, & Darnell JE, *Science* 264, 95-98, 1994.
2. Thompson JE et al., *Bioorg. Med. Chem. Lett.* 12, 2002.

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