

## Signal Transducer and Activator of Transcription 3 (STAT3) Antagonist Redistribution<sup>®</sup> 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. Another receptor ligand capable of activating STAT3 is the epidermal growth factor (EGF) that upon binding to the EGFR activates the STAT signalling pathway directly or indirectly via JAKs [2].

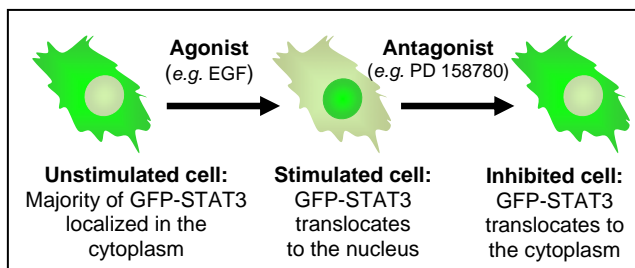


Figure 1: Illustration of the STAT3 translocation event.

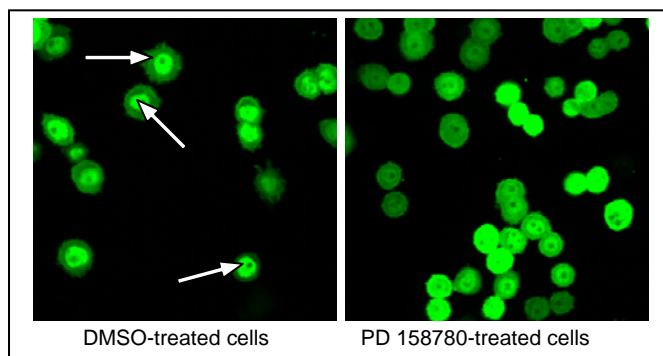


Figure 2: Images illustrating EGF-treated cells in the absence (DMSO) or presence of PD 158780. Arrows indicate EGF-mediated nuclear translocation detected by the image analysis algorithm.

The STAT3 antagonist Redistribution<sup>®</sup> 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 EGF is used as the reference agonist compound and test compounds are assayed for their ability to inhibit EGF-induced cytoplasm-to-nucleus translocation of STAT3. EGF-induced STAT3 nuclear translocation can be inhibited by the EGFR inhibitor PD 158780, thereby blocking STAT3 phosphorylation and subsequent nuclear import. In this assay, PD 158780 is used as a reference antagonist [3], with an EC<sub>50</sub>-value of about 15 nM. The EGFR inhibitor BPIQ-II has an EC<sub>50</sub>-value of about 10 nM in this assay [4].

Compounds inhibiting EGF-induced translocation could be interfering directly with STAT3 translocation, acting upstream of STAT3, or could be general nuclear import inhibitors/nuclear export activators. Since JAK inhibitors have no activity in this assay, the assay is suitable for distinguishing JAK inhibitors from compounds directly inhibiting STAT3 nuclear import when compounds are run in parallel in this EGF-mediated STAT3 assay as well as in our interleukin-6-mediated STAT3 Redistribution<sup>®</sup> assay [5].

Figures 1 and 2 illustrate the translocation event and Figure 3 illustrates the timelines involved in the STAT3 assay. Cells are incubated for 30 minutes with test compound in medium containing 5% serum before a 30-minute EGF treatment. Cells are fixed and stained with a nuclear counter-stain before the assay response is read in the INCell 3000 Analyzer (GE Healthcare). Compounds that antagonize the EGF-stimulated cytoplasm-to-nucleus translocation of STAT3 are positive in the assay, and the degree of inhibition is calculated as percent activity (PCTACT) relative to the PD 158780 control.

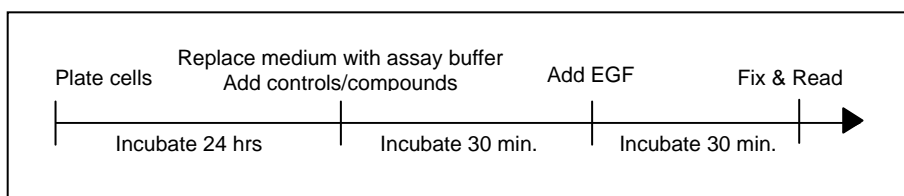


Figure 3: Summary of the assay timeline.



## Assay details

Cell line: MDA-MB-468 PS1508 GS (GFP-STAT3) grown in RPMI 1640 with Glutamax-1 containing 0.5 mg/ml Geneticin (G418), 1% (v/v) Penicillin/Streptomycin, and 10% FBS. Table 1 shows the final concentrations of DMSO, serum, EGF, and PD 158780 in the assay.

Assay	DMSO (%)	Serum (%)	EGF	PD 158780 ( $\mu\text{M}$ )
STAT3	0.25%	5% (0.5% BSA)	100 ng/ml	1

Table 1: Final DMSO, serum, EGF, and PD 158780 concentrations.

## Test compound handling for profiling services

Test compounds are stored at 4°C until diluted in neat DMSO. DMSO solutions are stored at -20°C. For preparation of the master concentration-response plates, neat DMSO is added to each test compound to give a final compound concentration of 25 mM. The master concentration-response plates are generated in half log dilutions corresponding to concentration response curves in the range 3.16 nM-31.6  $\mu\text{M}$ .

## Concentration response curve of the assay reference compound PD 158780

Figure 4A shows a concentration response curve of the reference compound, PD 158780 in the STAT3 assay. The  $\text{EC}_{50}$ -value of PD 158780 in the assay lies within the nano-molar range (*i.e.* ~15 nM). The  $\text{EC}_{50}$  value for BPIQ-II in the STAT3 assay is approximately 10 nM (Figure 4B).

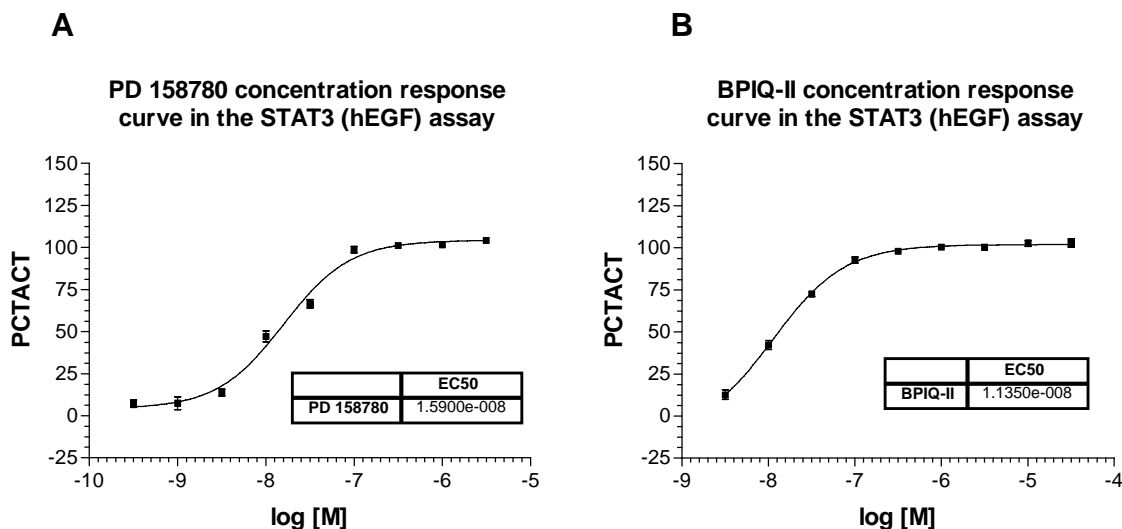


Figure 4: **A)** PD 158780 concentration response curve in the STAT3 assay, n=6. **B)** BPIQ-II concentration response curve in the STAT3 assay, n=6.

## References:

- Zhong Z, Wen Z, & Darnell JE, *Science* 264, 95-98, 1994
- Garcia R *et al.*, *Oncogene* 20, 2499-2513, 2001.
- Fry DW *et al.* *Biochem Pharmacol.* 54, 877-887, 1997.
- Rewcastle GW *et al.* *J. Med. Chem.* 39, 918-928, 1996.
- Biolmage, *STAT3 (IL6) Assay Application Note* 07-04-015.

## Notes:

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- The practice of translocation assays as described herein might violate Biolmage patents US 6,518,021; EP 0,986,753; US 6,172,188; EP 0,851,874 as well as other pending and granted patents.
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- The STAT3 assay in U2OS cells is available from Biolmage; the assay in BHK-21 cells is available for profiling and screening at Biolmage; it is available for purchase from GE Healthcare ([www.gehealthcare.com](http://www.gehealthcare.com)).