

CellSensor® SIE-*bla* ME-180 Cell Line

Cat. no. K1648

This cell-based assay has been thoroughly tested and validated by Invitrogen and is suitable for immediate use in a screening application. The following information illustrates the high level of assay testing completed and the validation of assay performance under optimized conditions.

Pathway Description

STAT family members, after being activated through the Jak/Stat pathway, form homo- or heterodimers that translocate to the cell nucleus where they act as transcription factors. STAT3 is activated through phosphorylation in response to various cytokines and growth factors including IFN γ , IL-6 and OSM. STAT3 homodimers and STAT3/STAT1 heterodimers interact with the sis-inducible element (SIE), a promoter sequence, thereby inducing transcription of genes related to cellular processes such as cell growth and apoptosis.

Cell Line Description

The CellSensor® SIE-*bla* ME-180 cell line contains a beta-lactamase reporter gene under control of the Sis-Inducible Element (SIE) stably integrated into ME-180 cells. ME-180 cells are human cervical carcinoma cells. This cell line has been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time and validated for Z' and EC₅₀ concentrations of human Interleukin 6 (hIL-6). Additional testing information using small molecule inhibitors and Stealth™ RNAi are also provided.

Validation Summary

Testing and validation of this assay was evaluated using LiveBLAZer™-FRET B/G Substrate.

1. Primary agonist dose response under optimized conditions

IL-6 EC ₅₀	= 0.6 ng/mL
Z'-Factor (EC ₁₀₀)	= 0.79
Response Ratio	= 19
Recommended cell no.	= 10K cells/well
Recommended [DMSO]	= up to 1%
Recommended Stim. Time	= 5 hours
Max. [Stimulation]	= ~33 ng/mL

2. Compound Panel

See *Compound Panel Section*

3. Small Molecule Inhibitor Testing

See *Small Molecule Inhibitor Section*

4. Stealth RNAi Testing

See *Stealth™ RNAi Testing Section*

5. Cell culture and maintenance

See *Cell Culture and Maintenance Section and Table 1*

Assay Testing Summary

6. Assay performance with variable cell number

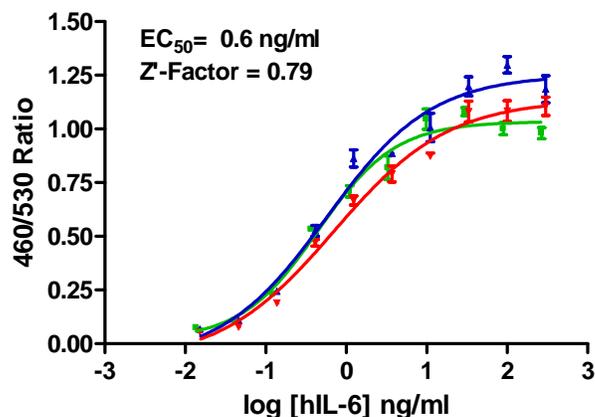
7. Assay performance with variable stimulation time

8. Assay performance with variable substrate loading time

9. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

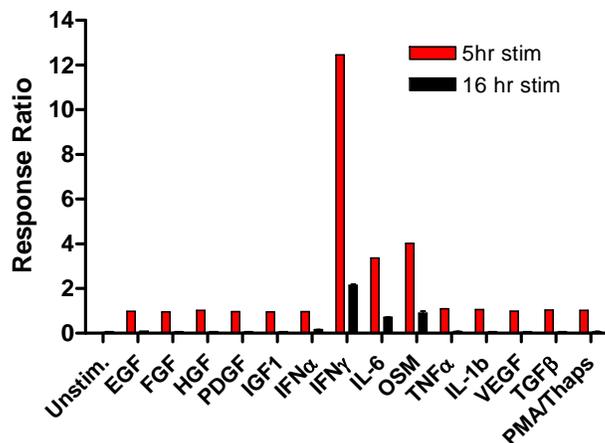
Figure 1 -SIE-*bla* ME-180 dose response to hIL-6 under optimized conditions



SIE-*bla* ME-180 cells (20,000 cells/well) were assayed on three separate days, represented by the three curves shown on the graph. Cells were plated in a 384-well plate (BD CellWare™ Poly-D-Lysine) and stimulated with hIL-6 (R&D Systems# IL-6) over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAZer™-FRET B/G Substrate for 2.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the 460/530 Ratios plotted against the indicated concentrations of hIL-6 (n=5 for each data point).

Compound Panel

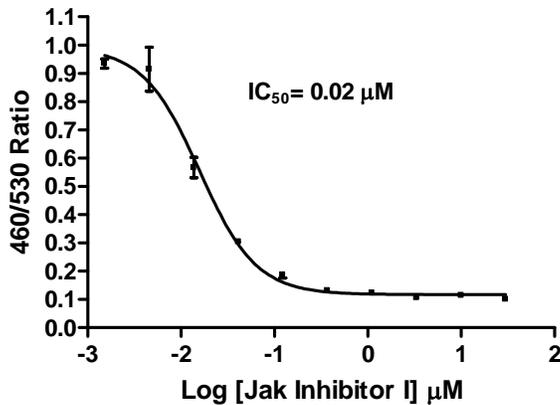
Figure 2 — SIE-*bla* ME-180 response to various compounds



SIE-*bla* ME-180 cells (50,000 cells/well) were plated in a 96-well plate and stimulated with the listed compounds in the presence of 0.5% DMSO for 5 or 16 hours. Cells were then loaded with LiveBLAZer™-FRET B/G Substrate for 2.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each compound (n=5 for each data point).

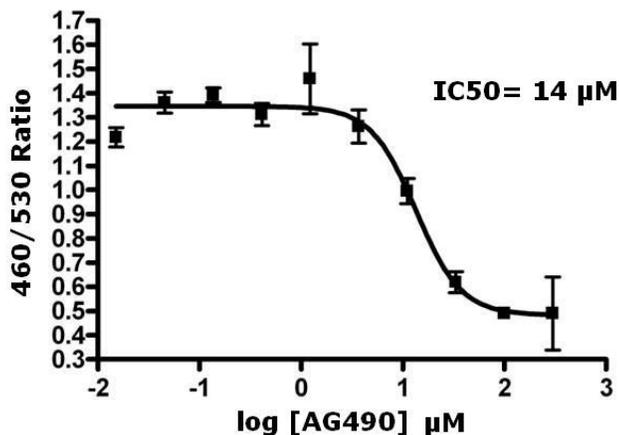
Small Molecule Inhibitor Testing

Figure 3 — SIE-*bla* ME-180 dose response to Jak Inhibitor I



SIE-*bla* ME-180 cells (50,000 cells/well) were plated in a 96-well plate and then treated with Jak Inhibitor I () for 1 hour, followed by stimulation with an EC_{80} concentration of hIL-6 (R&D Systems # 206-IL) in the presence of 0.5% DMSO for 5 hours. Cells were loaded with LiveBLAzer™-FRET B/G Substrate for 2.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of inhibitor (n=5 for each data point).

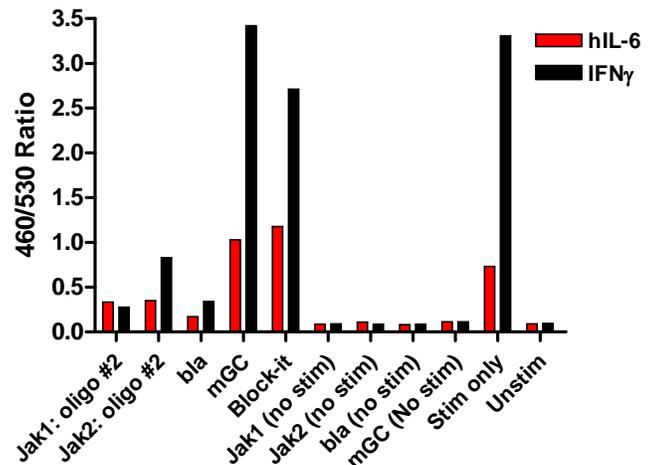
Figure 4 — SIE-*bla* ME-180 dose response to AG490



SIE-*bla* ME-180 cells (50,000 cells/well) were plated in a 96-well plate and then treated with AG490 (Calbiochem # 658-401) for 1 hour, followed by stimulation with an EC_{80} concentration of hIL-6 (R&D Systems # 206-IL) in the presence of 0.5% DMSO for 5 hours. Cells were loaded with LiveBLAzer™-FRET B/G Substrate for 2.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of inhibitor.

Stealth™ RNAi Testing

Figure 5 — SIE-*bla* ME-180 response to various RNAis



SIE-*bla* ME-180 cells (50,000 cells/well) were plated in a 96-well plate and then treated with Jak Inhibitor I () for 1 hour, followed by stimulation with an EC_{80} concentration of hIL-6 (R&D Systems # 206-IL) in the presence of 0.5% DMSO for 5 hours. Cells were loaded with LiveBLAzer™-FRET B/G Substrate for 2.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of inhibitor (n=5 for each data point).

Cell Culture and Maintenance

Thaw cells in Growth Medium without Blasticidin and culture them in Growth Medium with Blasticidin. Passage or feed cells at least twice a week and maintain them in a 37°C/5% CO₂ incubator. Maintain cells between 10% and 90% confluency. Do not allow cells to reach confluence.

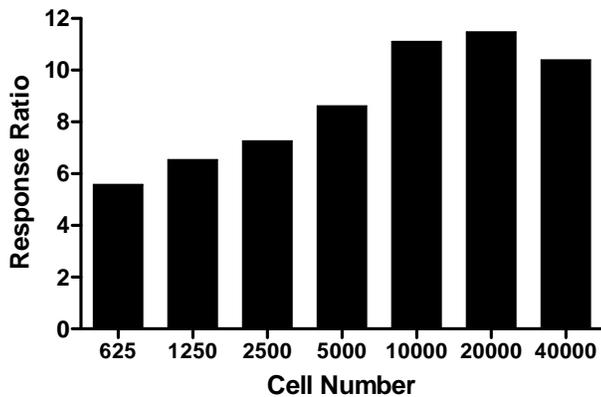
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For optimal cell line performance, use dialyzed FBS (Invitrogen # 26400-036).

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	Freezing Medium
DMEM	90%	—	—
Opti-MEM®	—	99.5%	—
Dialyzed FBS Do not substitute!	10%	0.5%	—
NEAA	0.1 mM	0.1 mM	—
Sodium pyruvate	—	1 mM	—
HEPES (pH 7.3)	25 mM	—	—
Penicillin (antibiotic)	100 U/ml	100 U/ml	—
Streptomycin (antibiotic)	100 µg/ml	100 µg/ml	—
Blasticidin (antibiotic)	5 µg/ml	—	—
Recovery™ Cell Culture Freezing Medium	—	—	100%

Assay Performance with Variable Cell Number

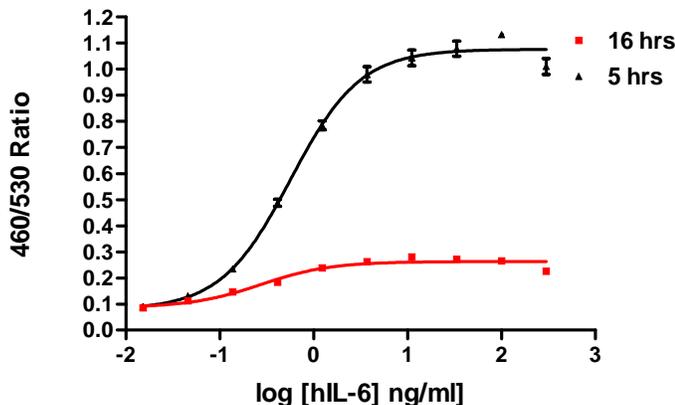
Figure 5 — SIE-*bla* ME-180 response to hIL-6 using 625, 1250, 2500, 5000, 10000, 20000, or 40000 cells/well



SIE-*bla* ME-180 cells were plated at ~625, ~1250, 2500, 5000, 10000, 20000, or 40,000 cells/well in a 384-well format. Cells were then stimulated with 100 ng/ml of hIL-6 (R&D Systems # 206-IL) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2.5 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios for each cell number plotted against the indicated concentrations of hIL-6.

Assay Performance with Variable Stimulation Time

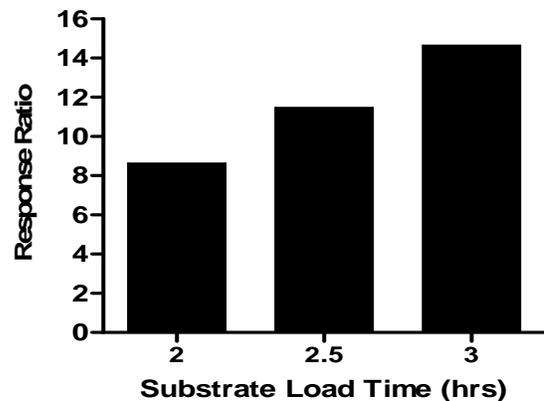
Figure 6 – SIE-*bla* ME-180 dose response to hIL-6 using 5 and 16 hour stimulation times.



SIE-*bla* ME-180 cells (20,000 cells/well) were plated in a 384-well assay plate. hIL-6 (R&D Systems # 206-IL) was then added to the plate over the indicated concentration range. Plates were stimulated for 5 or 16 hrs with hIL-6 in 0.5% DMSO and then loaded for 2.5 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the 460/530 Ratios plotted for each stimulation time against the indicated concentrations of agonist (n=5 for each data point).

Assay Performance with Variable Substrate Loading Time

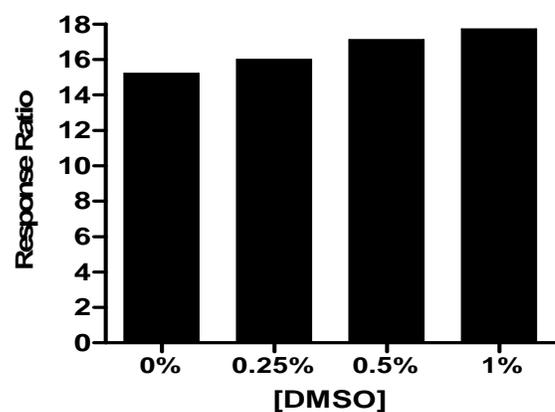
Figure 7 — SIE-*bla* ME-180 response to hIL-6 with 1, 1.5, 2 and 2.5 hour substrate loading times



SIE-*bla* ME-180 cells were plated at 20,000 cells/well in a 384-well format. Cells were stimulated with 100 ng/ml of hIL-6 (R&D Systems# 206-IL) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1, 1.5, 2 or 2.5 hours. Fluorescence emission values at 460 nm and 530 nm for the various substrate loading times were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each substrate loading time.

Assay Performance with Variable DMSO Concentration

Figure 8 – SIE-*bla* ME-180 response to hIL-6 using 0, 0.25, 0.5 and 1% DMSO



SIE-*bla* ME-180 cells (20,000 cells/well) were plated in a 384-well plate and treated with 100 ng/ml hIL-6 (R&D Systems # 206-IL) with final DMSO concentrations ranging from 0% to 1%. Plates were stimulated for 5 hrs and loaded for 2.5 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each DMSO concentration.