

CellSensor® SIE-*bla* HEK 293T Cell Line

Cat. no. K1649

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* HEK 293T cell line contains a beta-lactamase reporter gene under control of the Sis-Inducible Element (SIE) stably integrated into HEK 293T cells. This cell line has been tested for assay performance under variable conditions, including DMSO concentration, stimulation time, substrate loading time and validated for Z' and EC₅₀ concentrations of human Interleukin 6 (hIL-6). Additional testing information using Interferon gamma (IFN γ) is 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(n=3)

hIL-6 EC₅₀ = 2.4 ng/mL
Z'-Factor (EC₁₀₀) = 0.88
Response Ratio = 14

Recommended cell no. = 20K cells/well
Recommended [DMSO] = up to 0.5%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = ~450 ng/mL

2. Alternate Agonist Dose Response

See *Alternate Agonist Testing Section*

3. Cell culture and maintenance

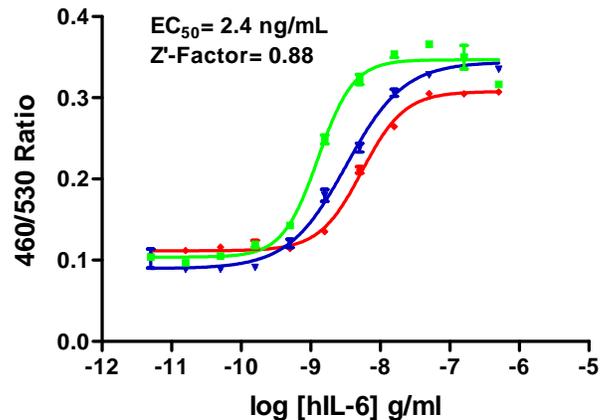
See *Cell Culture and Maintenance Section and Table 1*

Assay Testing Summary

4. Assay performance with variable stimulation time
5. Assay performance with variable substrate loading time
6. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

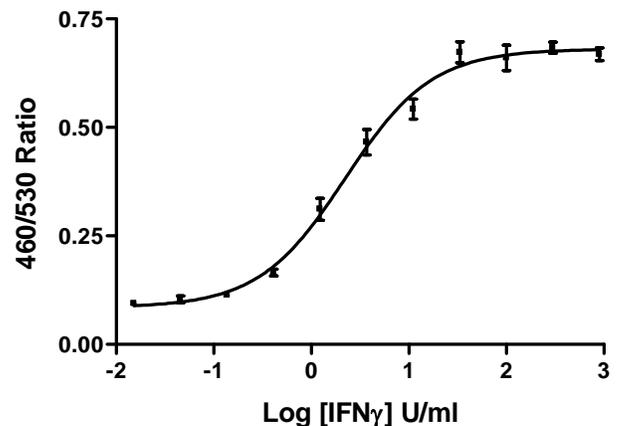
Figure 1 -SIE-*bla* HEK 293T dose response to hIL-6 under optimized conditions



SIE-*bla* HEK 293T 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 and stimulated with human IL-6 (Calbiochem # 407652) 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 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).

Alternate Agonist Dose Response

Figure 2 — SIE-*bla* HEK 293T dose response to IFN γ



SIE-*bla* HEK 293T cells (50,000 cells/well) were plated in a 96-well plate and stimulated with IFN γ (Biosource #PHC4031) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 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 agonist (n=4 for each data point).

Cell Culture and Maintenance

Thaw cells in Growth Medium without Blastcidin and culture them in Growth Medium with Blastcidin. 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.

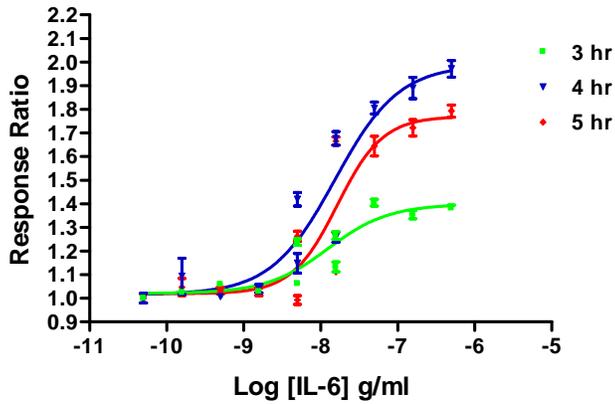
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For optimal cell line performance, use dialyzed FBS (Invitrogen # 26400-036).

Table 1 – Cell Culture and Maintenance

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

Assay Performance with Variable Stimulation Time

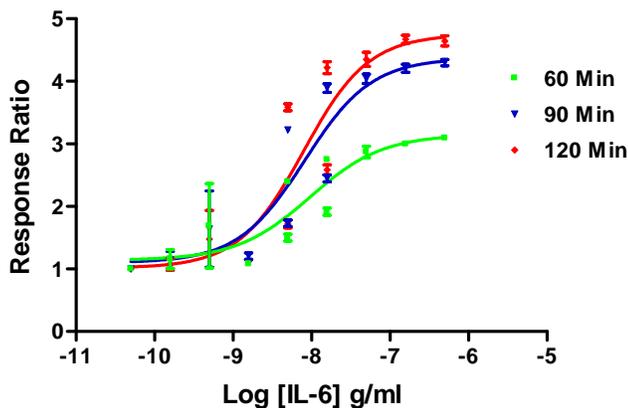
Figure 3 – SIE-*bla* HEK 293T dose response to hIL-6 using 3, 4 and 5 hour stimulation times



SIE-*bla* HEK 293T cells (20,000 cells/well) were plated in a 384-well assay plate. Human IL-6 (Calbiochem #407652) was then added to the plate over the indicated concentration range. Plates were stimulated for 3, 4 or 5 hrs with IL-6 in 0.5% DMSO and then loaded for 2 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 for each stimulation time plotted against the indicated concentrations of agonist (n=8 for each data point).

Assay Performance with Variable Substrate Loading Time

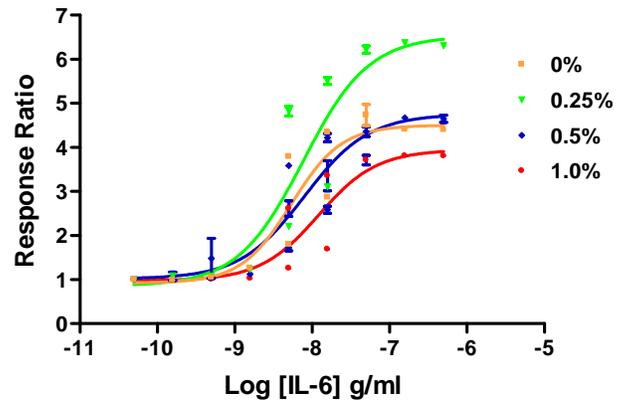
Figure 4 – SIE-*bla* HEK 293T response to hIL-6 with 1, 1.5, and 2 hour substrate loading times



SIE-*bla* HEK 293T cells were plated at 20,000 cells/well in a 384-well format. Cells were stimulated with 100 ng/ml of hIL-6 (Calbiochem #407652) 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, or 2 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 of each substrate loading time plotted against the indicated concentrations of hIL-6 (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 5 – SIE-*bla* HEK 293T response to hIL-6 using 0, 0.25, 0.5 and 1% DMSO



SIE-*bla* HEK 293T cells (20,000 cells/well) were plated in a 384-well plate and treated with the indicated concentrations of hIL-6 (Calbiochem #407652) with final DMSO concentrations ranging from 0% to 1%. Plates were stimulated for 5 hrs and loaded for 2 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 for each DMSO concentration plotted against the indicated concentrations of agonist (n=8 for each data point).