Validation & Assay Performance Summary

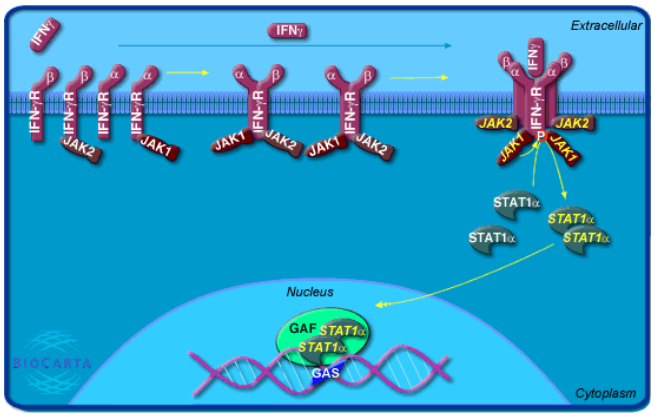
CellSensor® SIE-*bla* THP-1 Cell Line

Cat. no. K1652

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

Interferon gamma (IFN-γ) plays a role in activating lymphocytes to enhance anti-microbial and anti-tumor effects. In response to IFN-γ stimulation, monocytes/macrophages produce cell surface molecules for antigen-presentation and proinflammatory cytokines. Blocking IFN-γ induced response provides a strategy for Inflammation therapy. Signaling takes place through binding of IFN-γ to an IFN Receptor complex consisting of two alpha chains (Type I receptor) and two beta chains (Type 2 receptor). Upon phosphorylation by Jak1/2, the transcription factor STAT 1 homodimerizes and translocates to the nucleus and binds to SIE/GAS and activates downstream gene expression.



Cell Line Description

The CellSensor® SIE-*bla* THP-1 cell line contains a beta-lactamase reporter gene under control of the SIE response element stably integrated into THP-1 cells. THP-1 cells are human acute monocytic leukemia cells. This cell line has also been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, substrate loading time, and validated for Z' and EC_{50} concentrations of IFN-γ. Additional testing information with a panel of ligands 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**

IFN-γ EC_{50}	= 1.93 ng/mL
Z'-Factor (EC_{100})	= 0.76
Response Ratio	= 15.47
Optimum cell no.	= 30K cells/well
Optimum [DMSO]	= 0.5%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 222.22 ng/mL

2. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

3. **Assay performance with variable cell number**

4. **Assay performance with variable stimulation time**

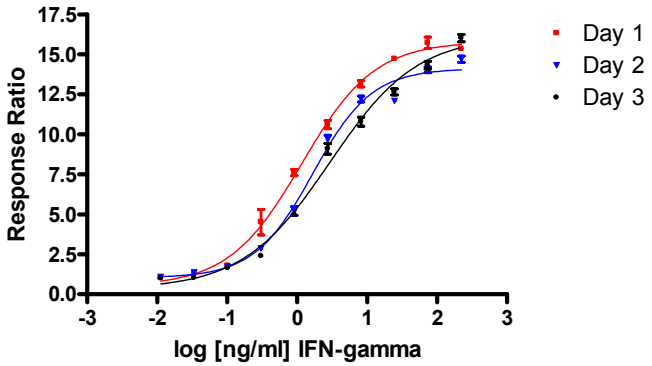
5. **Assay performance with variable substrate loading time**

6. **Assay performance with variable DMSO concentration**

7. **Ligand Panel**

Primary Agonist Dose Response

Figure 1 –SIE-*bla* THP-1 dose response to IFN-γ under optimized conditions



SIE-*bla* THP-1 cells (30,000 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 IFN-γ 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 Response Ratios plotted for the indicated concentrations of IFN-γ (n=16 for each data point).

Cell Culture and Maintenance

Thaw cells in Growth Medium without Blasticidin and culture them in Growth Medium with Blasticidin. Pass or feed cells at least twice a week and maintain them in a 37°C/5% CO_2 incubator. Maintain cells between 10^5 and 10^6 cells/ml. Do not allow cells to reach confluence

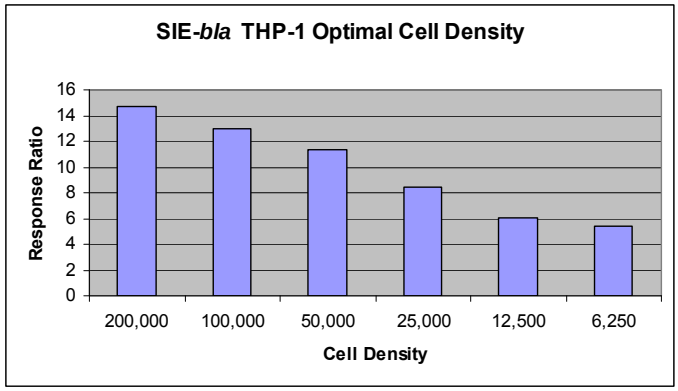
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. Freeze cells at 5 x 10^6 cells/ml in Freezing Medium. For optimal cell line performance, use Dialyzed FBS (Invitrogen # 26400-036). For detailed growth and maintenance directions, please refer to the protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	Freezing Medium
RPMI 1640	90%	—	80%
Opti–MEM® 1	—	99.5%	—
Dialyzed FBS Do not substitute!	10%	0.5%	10%
NEAA	0.1 mM	0.1 mM	0.1 mM
Sodium pyruvate	1 mM	1 mM	1 mM
Penicillin (antibiotic)	100 U/ml	100 U/ml	100 U/ml
Streptomycin (antibiotic)	100 µg/ml	100 µg/ml	100 µg/ml
Blasticidin (antibiotic)	5 µg/ml (do not thaw with Blasticidin)	—	—
DMSO	—	—	10%

Assay Performance with Variable Cell Number

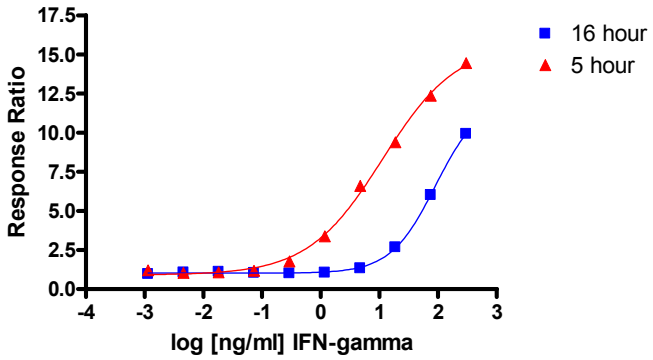
Figure 2 — SIE-*bla* THP-1 response to IFN-γ using 6,250, 12,500, 25,000, 50,000, 100,000, or 200,000 cells/well



SIE-*bla* THP-1 cells were plated at 6,250, 12,500, 25,000, 50,000, 100,000 or 200,000 cells/well in a 384-well format. Cells were then stimulated with IFN-γ at 150 ng/ml 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 plotted for each cell number against the fixed concentration of IFN-γ.

Assay Performance with Variable Stimulation Time

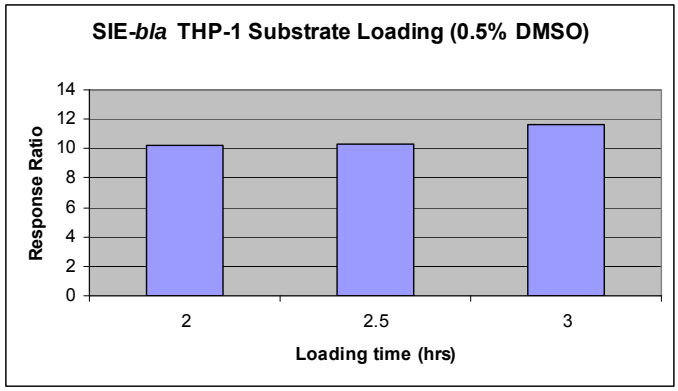
Figure 3 – SIE-*bla* THP-1 dose response to IFN-γ using 5 and 16 hour stimulation times



SIE-*bla* THP-1 cells (30,000 cells/well) were plated the in a 384-well assay plate. IFN-γ was then added to the plate at over the indicated concentration range. Plates were stimulated for 5 or 16 hrs with IFN-γ 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 Response Ratios plotted for each stimulation time.

Assay Performance with Variable Substrate Loading Time

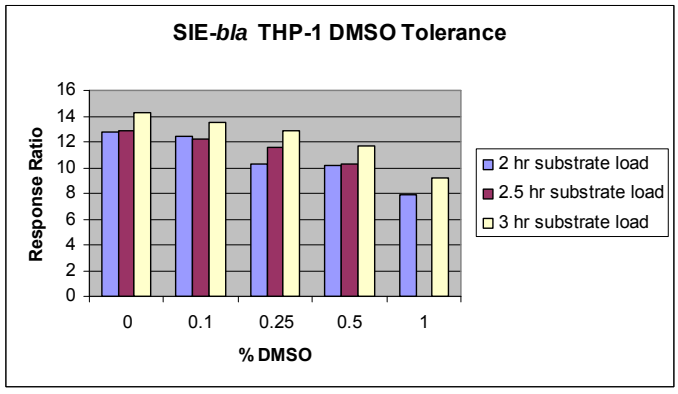
Figure 4 — SIE-*bla* THP-1 response to IFN-γ with 2, 2.5 and 3 hour substrate loading times



SIE-*bla* THP-1 cells were plated at 30,000 cells/well in a 384-well format. Cells were stimulated with 150 ng/ml IFN-γ in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 2, 2.5 or 3 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 the indicated substrate loading times.

Assay Performance with Variable DMSO Concentration

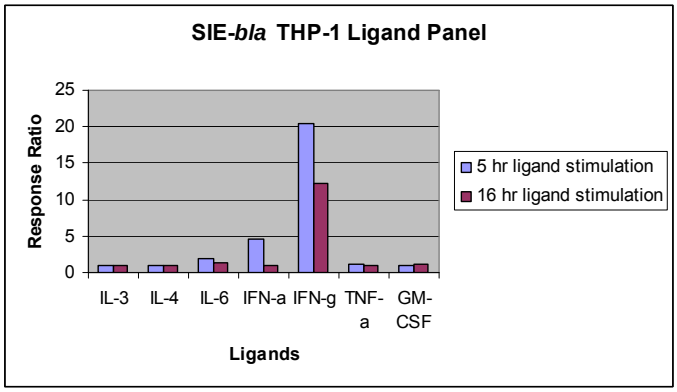
Figure 5 –SIE-*bla* THP-1 response to IFN-γ using 0, 0.1, 0.25, 0.5 and 1% DMSO



SIE-*bla* THP-1 cells (30,000 cells/well) were plated in a 384-well plate and treated with the indicated concentrations of IFN-γ 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. (Data was not obtained for 2.5 hour substrate loading time, 1% DMSO)

Ligand Panel Results

Figure 6 — SIE-*bla* THP-1 response to various ligands with 5 and 16 hour stimulation times



SIE-*bla* THP-1 cells were plated at 100,000 cells/well in a 96-well format. Cells were stimulated with various ligands 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each ligand tested.