Validation & Assay Performance Summary

CellSensor® GAS-*bla* ME180 Cell Line

Cat. no. K1651

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 a 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® GAS-*bla* ME180 cell line contains a beta-lactamase reporter gene under control of the GAS promoter stably integrated into ME180 cells. This cell line is a clonal population isolated in response to IFN-γ by flow cytometry. 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}	= 5.98 ng/mL
Z'-Factor (EC_{100})	= 0.79
Response Ratio	= 7.07
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= 0.5%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 300 ng/mL

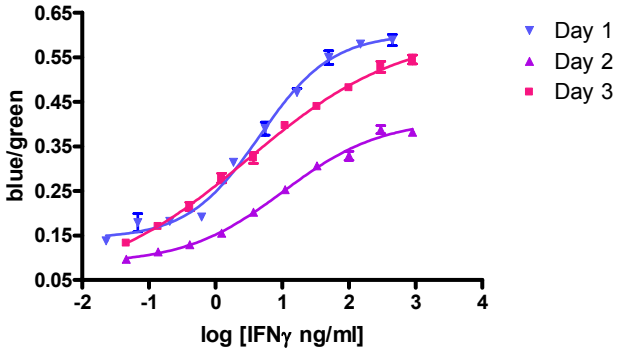
2. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

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**
8. **RNAi testing**

Primary Agonist Dose Response

Figure 1 –GAS-*bla* ME180 dose response to IFN-γ under optimized conditions



GAS-*bla* ME180 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 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% and 90% confluency. Do not allow cells to reach confluence

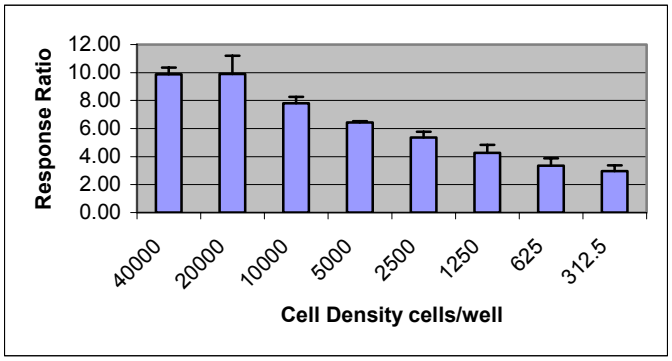
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. Freeze cells at 2 x 10^6 cells/ml in Freezing Medium. For optimal cell line performance, use Dialyzed FBS (Invitrogen # 26400-036). For detailed growth and maintenance directions, please refer to the protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	Freezing Medium
DMEM	90%	—	—
Opti–MEM® 1	—	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	10 mM	—
Penicillin (antibiotic)	100 U/ml	100 U/ml	—
Streptomycin (antibiotic)	100 µg/ml	100 µg/ml	—
Blasticidin (antibiotic)	5 µg/ml (do not thaw with Blasticidin)	—	—
Cell Culture Freezing Medium	—	—	100%

Assay Performance with Variable Cell Number

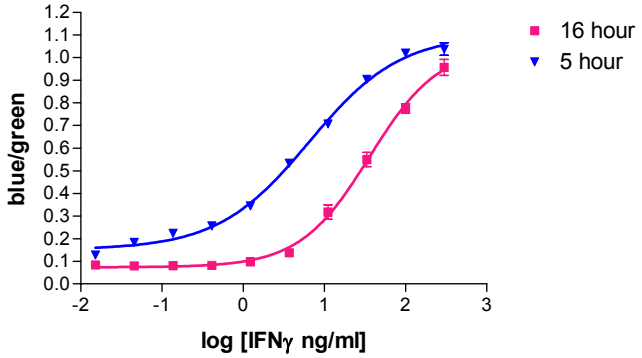
Figure 2 — GAS-*bla* ME180 response to IFN-γ using 312.5, 625, 1,250, 2,500, 5,000, 10,000, 20,000 and 40,000 cells / well



GAS-*bla* ME180 cells were plated at 312.5, 625, 1,250, 2,500, 5,000, 10,000, 20,000 and 40,000 cells/well in a 384-well format. Cells were then stimulated with IFN-γ at 50 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

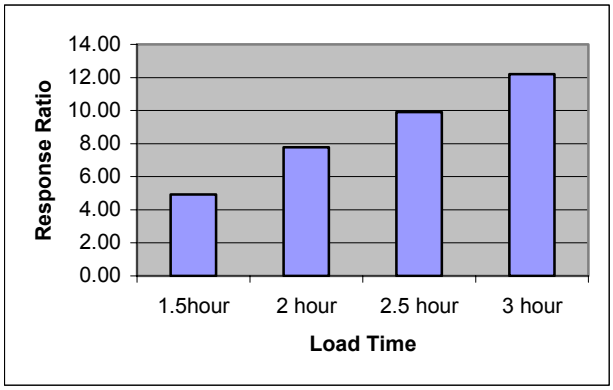
Figure 3 – GAS-*bla* ME180 dose response to INF-γ using 5 and 16 hour stimulation times



GAS-*bla* ME180 cells (20,000 cells/well) were plated the in a 384-well assay plate. 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

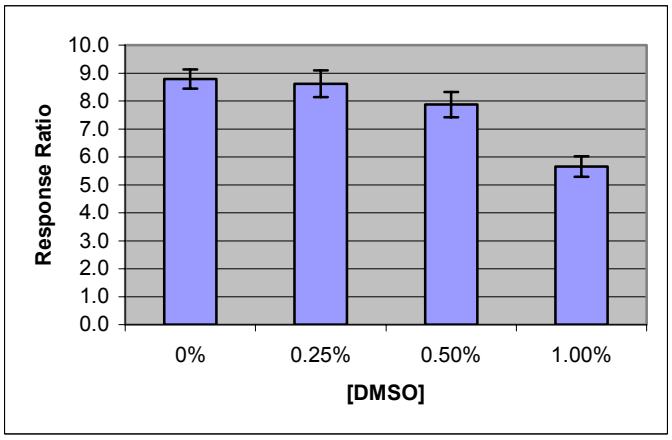
Figure 4 — GAS-*bla* ME180 response to IFN-γ with 1.5, 2, 2.5 and 3 hour substrate loading times



GAS-*bla* ME180 cells were plated at 20,000 cells/well in a 384-well format. Cells were stimulated with 100 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 1.5, 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

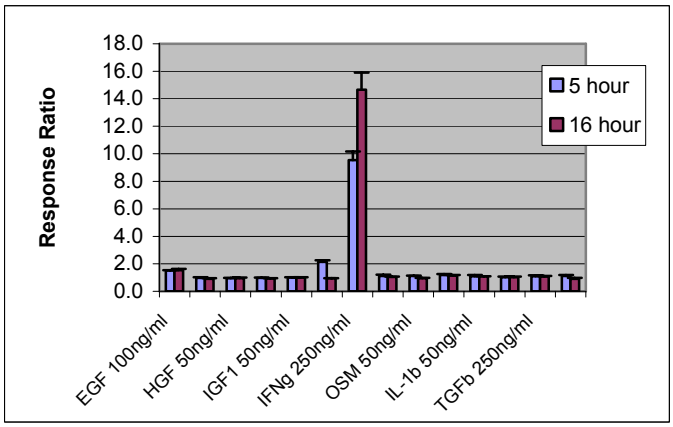
Figure 5 – GAS-*bla* ME-180 response to IFN-γ using 0, 0.25, 0.5 and 1% DMSO



GAS-*bla* ME180 cells (20,000 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.

Ligand Panel Results

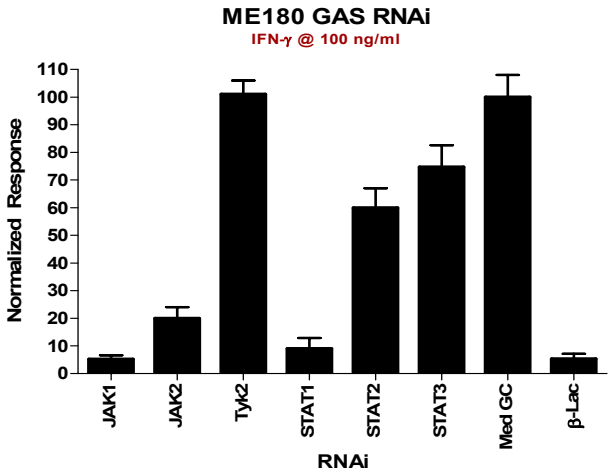
Figure 6 — GAS-*bla* ME180 response specifically to IFNγ with 5 and 16 hour stimulation times



GAS-*bla* ME180 cells were plated at 20,000 cells/well in a 384-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.

RNAi Testing Results

Figure 7 — GAS-*bla* ME180 response to IFN-γ can be knocked down by Jak1, Jak2 and Stat1 specific RNAi oligos



GAS-*bla* ME180 cells (8,000 cells/well) were plated in a 96-well plate and incubated with indicated Stealth™ RNAi for 48 hours (Invitrogen, JAK1 (12937-45), JAK2 (12937-28), STAT1 (12936-65). Plates were stimulated for 5 hrs with 100ng/ml IFN-γ 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 Normalized Response plotted for each RNAi condition.