Validation & Assay Performance Summary

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

Cat. no. K1655

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

Interferons and their signaling pathways are known to be involved in anti-viral defense and auto-immune responses. Some interferons are also used in novel cancer treatments. The Interferon Alpha A signaling pathway, which includes members of the JAK(Janus kinase) family of kinases and the STAT(Signal Transducer and Activator of Transcription) family of transcription factors. Interferon Alpha A binds to its cognate receptor on the cell surface. JAK1 and TYK2 are then activated which in turn activate STAT 1 and 2. These STATs form a heterodimer and bind an additional 48kDa protein. This STAT1/STAT2/48kDa protein complex, referred to as ISGF-3(Interferon Stimulated Gene Factor-3), enters the nucleus and binds a region of DNA called ISRE (Interferon Stimulated Response Element) to activate transcription.

Cell Line Description

The CellSensor® ISRE-*bla* HEK 293T cell line contains a beta-lactamase reporter gene under control of the Interferon Stimulated Response Element (ISRE) stably integrated into HEK 293T cells. This cell line validated for EC_{50} and Z'-Factor under optimized conditions using Interferon alpha (IFNα). This cell line has also been tested under variable experimental conditions, including cell number, stimulation time, and substrate loading time. Small molecule inhibitor and Stealth™ RNAi testing data is also provided.

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Primary agonist dose response under optimized conditions(n=3)**

Interferon alpha IC_{50}	= 278 U/ml
Z'-Factor (EC_{100})	= 0.93
Response Ratio	= 7
Optimum cell no.	= 25K cells/well
Optimum [DMSO]	= 0.25%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= ~8000 U/ml

2. **Small Molecule Inhibitor Testing**
   See small molecule inhibitor testing section

3. **RNAi testing**
   See RNAi testing section

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

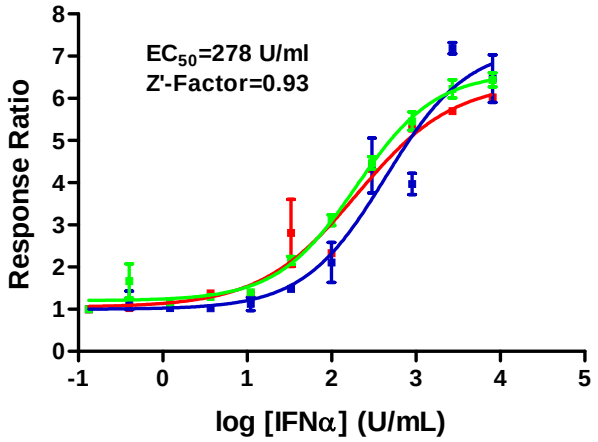
Assay Testing Summary

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

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

Primary Agonist Dose Response

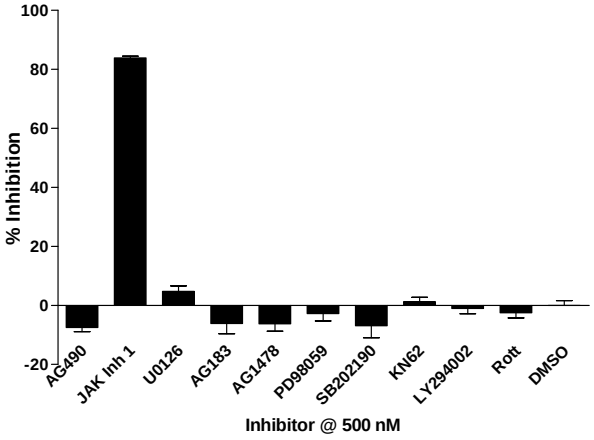
Figure 1 —ISRE-*bla* HEK 293T dose response to Interferon alpha (IFNα) under optimized conditions



ISRE-*bla* HEK 293T cells (25,000 cells/well) were assayed on three separate days represented by the three curves shown on the graph. Cells were plated the day of the assay in a 384-well format and stimulated with Interferon alpha (R&D Systems # 11100-1) over the indicated concentration range in the presence of 0.1% 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 Emission Ratios plotted for the indicated concentrations of Interferon alpha (n=16 for each data point).

Small Molecule Inhibitor Testing

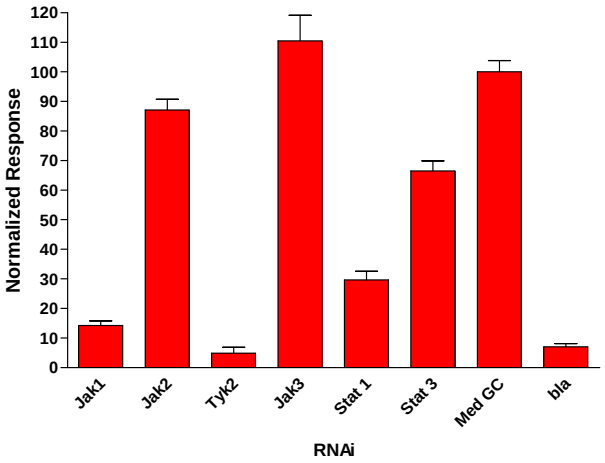
Figure 2 —ISRE-*bla* HEK 293T response to various small molecule inhibitors



ISRE-*bla* HEK 293T cells (50,000 cells/well) were plated the day of the assay in a 96-well format. Cells were treated with the listed inhibitors at 500 nM final concentration for 1 hr followed by treatment with 300 U/ml Interferon alpha (R&D Systems # 11100-1) for 5 hours. Cells were then loaded with LiveBLAzer™ -FRET B/G Substrate for 2 hours. Fluorescence emission values were obtained using a standard fluorescence plate reader and converted to % inhibition.

RNAi Testing

Figure 3 — ISRE-bla HEK 293T response to various Stealth™ RNAis



ISRE-*bla* HEK 293T cells (8,000 cells/well) were plated the day of the assay in a 96-well format. Cells were treated with Lipofectamine™ 2000 mixtures containing the listed Stealth™ RNAi oligos for 60 hrs, followed by treatment with 300 U/ml Interferon alpha (R&D Systems # 11100-1) for 5 hours. Cells were then loaded with LiveBLAzer™ -FRET B/G Substrate for 2 hours. Fluorescence emission values were obtained using a standard fluorescence plate reader and converted to a normalized response.

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.

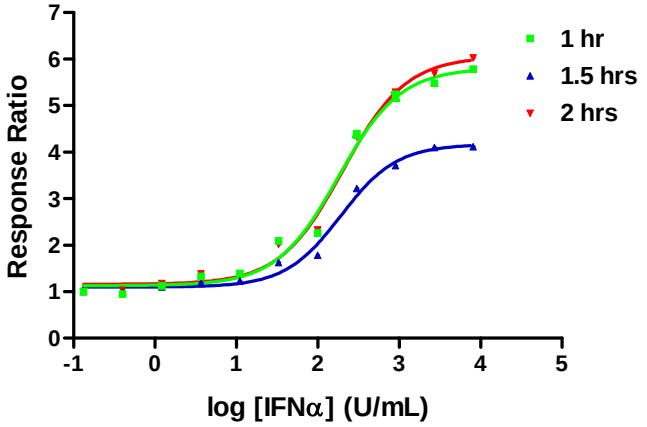
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For proper cell line performance, use dialyzed FBS (Invitrogen# 26400-036). For more detailed cell growth and maintenance directions, please refer to protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium (-)	Growth Medium (+)	Assay Medium
DMEM	90%	90%	90%
Dialyzed FBS	10%	10%	10%
NEAA	0.1mM	0.1mM	0.1mM
Sodium Pyruvate	1mM	1mM	1mM
HEPES (pH 7.0)	25 mM	25 mM	25 mM
Penicillin	--	100 U/ml	100 U/ml
Streptomycin	--	100 µg/ml	100 µg/ml
Blasticidin	--	5 µg/ml	--

Assay Performance with Variable Substrate Loading Time

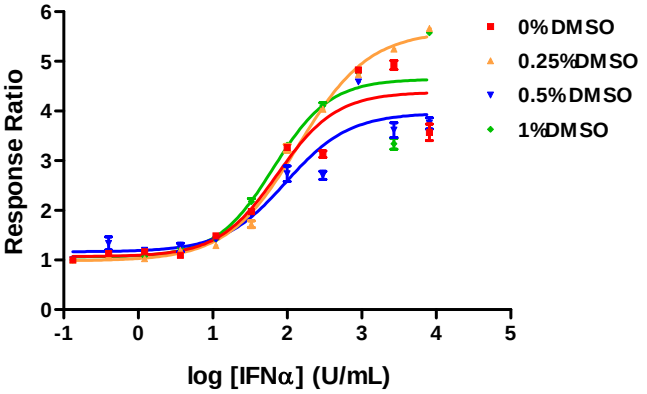
Figure 4 — ISRE-*bla* HEK 293T dose response to Interferon alpha with 1, 1.5, and 2 hour substrate loading times



ISRE-*bla* HEK 293T cells were plated the day of the assay at 25,000 cells/well in a 384-well format. Cells were treated with Interferon alpha (R&D Systems # 11100-1) over the indicated concentration range in the presence of 0.1% 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 were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each substrate loading time against the indicated concentrations of Interferon alpha (n=8 for each data point).

Assay Performance with Variable DMSO

Figure 5 – ISRE-*bla* HEK 293T dose response to Interferon alpha with 0, 0.25, 0.5 and 1% DMSO



ISRE-*bla* HEK 293T cells were plated the day of the assay at 25,000 cells/well in a 384-well format. Cells were treated with Interferon alpha (R&D Systems # 11100-1) over the indicated concentration range for 5 hours with final DMSO concentrations of 0, 0.25, 0.5 or 1%. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 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 DMSO concentration plotted against indicated concentrations of Interferon alpha (n=8 for each data point).