Validation & Assay Performance Summary

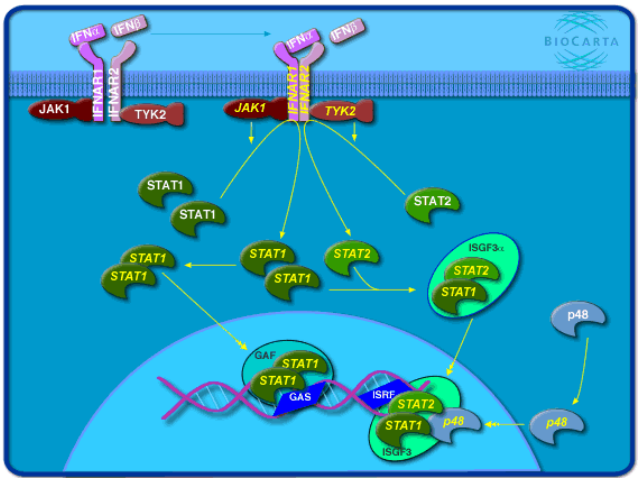
CellSensor® ISRE-*bla* Jurkat Cell Line

Cat. no. K1656

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 alpha plays a role in viral infections. Signaling takes place through an IFN Receptor complex consisting of two alpha chains (Type I receptor) complexed with Jak1 and Tyk2. These kinases phosphorylate Stat1 and Stat2, which then form heterodimers, translocate to the nucleus and bind to ISRE (Interferon Stimulated Response Element) and induce downstream gene expression.



Cell Line Description

The CellSensor® ISRE-*bla* Jurkat cell line contains a beta-lactamase reporter gene under control of the ISRE response element stably integrated into Jurkat cells. This cell line is a clonal population isolated in response to IFNα 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 forskolin. Responsiveness to 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**

IFNα EC_{50}	= 40.53 U/ml
Z'-Factor (EC_{100})	= 0.83
Response Ratio	= 5.66
Optimum cell no.	= 30K cells/well
Optimum [DMSO]	= 0.5%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 3000 U/ml

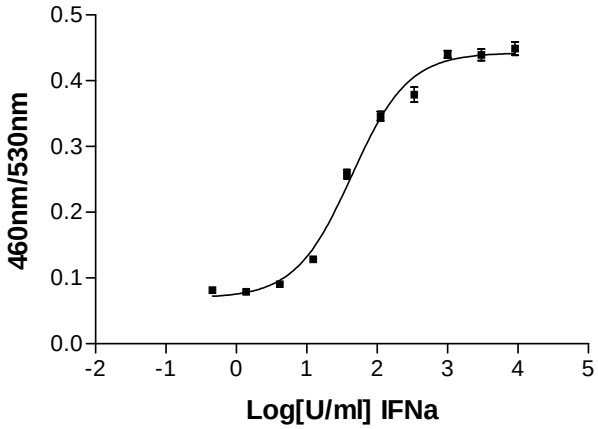
2. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

3. **Assay performance with variable cell number**
4. **Assay performance with variable substrate loading time**
5. **Assay performance with variable DMSO concentration**
6. **Ligand panel**

Primary Agonist Dose Response

Figure 1 –ISRE-*bla* Jurkat dose response to IFNα under optimized conditions



ISRE-*bla* Jurkat cells (30,000 cells/well) were plated in a 384-well plate and stimulated with IFNα 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 3.5 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 IFNα (data is average of data collected on three separate days).

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 $0.5x10^5$ and $1.5x10^6$ cells per well. Do not allow cells to reach confluence.

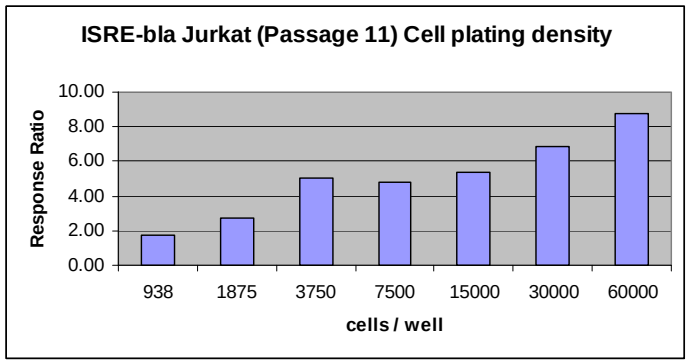
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. Freeze cells at 5 x 10^6 cells/ml in Freezing Medium. For optimal cell line performance, use Dialyzed FBS (Invitrogen # 26400-036). For detailed growth and maintenance directions, please refer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

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	Freezing Medium
RPMI 1640	90%	—	80%
Opti-MEM®	—	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
HEPES (pH 7.3)	—	10 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

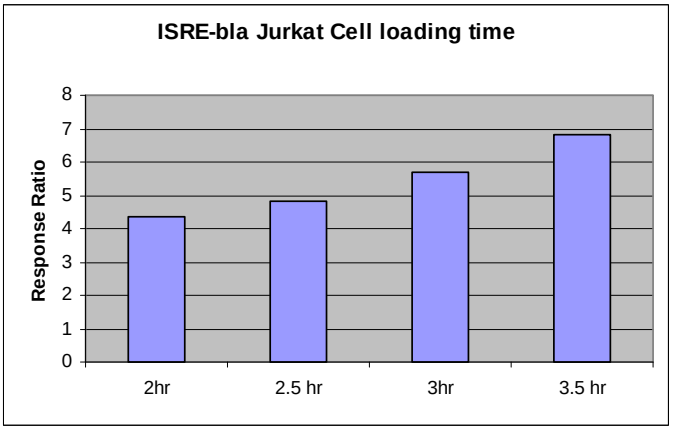
Figure 2 - ISRE-*bla* Jurkat response to IFNα using 938, 1,875, 3,750, 7,500, 15,000, 30,000, and 60,000 cells/well



ISRE-*bla* Jurkat cells were plated at 938, 1,875, 3,750, 7,500, 15,000, 30,000, and 60,000 cells/well in a 384-well format. Cells were then stimulated with IFNα at various concentrations in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 3.5 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.

Assay Performance with Variable Substrate Loading Time

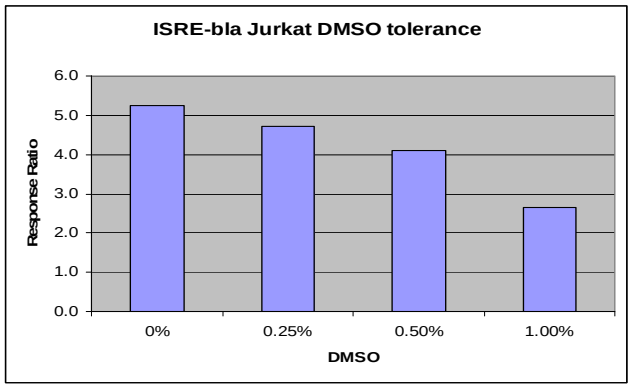
Figure 3 — ISRE-*bla* Jurkat response to IFNα with 2, 2.5, 3 and 3.5 hour substrate loading times



ISRE-*bla* Jurkat cells were plated at 30,000 cells/well in a 384-well format. Cells were stimulated with IFNα at various concentrations 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, 3 or 3.5 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 the indicated substrate loading times.

Assay Performance with Variable DMSO Concentration

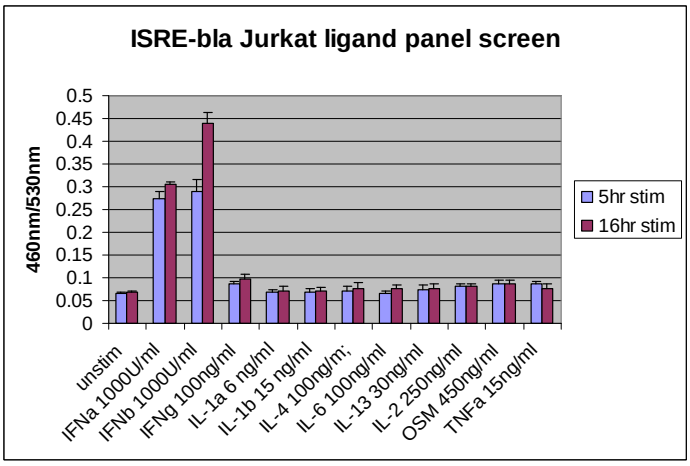
Figure 4 – ISRE-*bla* Jurkat response to IFNα using 0, 0.25, 0.5 and 1% DMSO



ISRE-*bla* Jurkat cells (30,000 cells/well) were plated in a 384-well plate and treated with the indicated concentrations of IFNα with final DMSO concentrations ranging from 0% to 1%. Plates were stimulated for 5 hrs and loaded for 3.5 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

Figure 5 – ISRE-*bla* Jurkat response to IFNα using 0, 0.25, 0.5 and 1% DMSO



ISRE-*bla* Jurkat cells (30,000 cells/well) were plated in a 384-well plate and treated with various ligands with final DMSO concentrations of 0.5%. Plates were stimulated for 5 hrs and loaded for 3.5 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 liand tested.