CellSensor® irf1-*bla* TF-1 Cell-based Assay Protocol

Catalog no. K1657 **Shipping: Dry Ice** **Storage: Liquid Nitrogen**

Protocol part no. K1219.pps Rev. date: 21 August 2009

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1. Overview of GeneBLAzer® Technology

GeneBLAzer® Beta-lactamase Reporter Technology provides a highly accurate, sensitive, and easy to use method of monitoring cellular responses to drug candidates or other stimuli (1). The core of the GeneBLAzer® Technology is a Fluorescence Resonance Energy Transfer (FRET) substrate that generates a ratiometric reporter response with minimal experimental noise. In addition to the two-color (blue/green) readout of stimulated and unstimulated cells, this ratiometric method reduces the absolute and relative errors that can mask the underlying biological response of interest. Such errors include variations in cell number, transfection efficiency, substrate concentration, excitation path length, fluorescence detectors, and volume changes. The GeneBLAzer® Beta-lactamase Reporter Technology has been proven effective in high-throughput screening (HTS) campaigns for a range of target classes, including G-protein coupled receptors (GPCRs) (2, 3), nuclear receptors (4, 5), and kinase signaling pathways (6).

2. Materials Supplied

Cell Line Name:	irf1-bla TF-1
Description:	CellSensor® irf1-*bla* TF-1 cells contain a beta-lactamase reporter gene under control of the STAT5 Response Elements present in the Interferon Regulatory Factor I (IRF1) gene promoter that has been stably integrated into TF-1 cells. CellSensor® irf1-*bla* TF-1 cells respond to Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), IL-3 and EPO.
Product Number:	K1657
Shipping Condition:	Dry Ice
Storage Condition:	Liquid nitrogen. Immediately upon receipt, cells must be stored in liquid nitrogen or thawed for immediate use. Cells stored at -80°C can quickly lose viability.
Quantity:	Refer to the Certificate of Analysis
Application:	Detection of agonists/antagonists of the JAK2/STAT5 signaling pathway.
Growth Properties:	Suspension, doubling time 20–30 hours,
Propagation:	Maintain between 30,000 and 700,000 cells/mL
Cell Phenotype:	Lymphoblast
Selection Marker:	Blasticidin (5 µg/mL)
Vector Used:	pLenti-bsd/irf1-bla
***Mycoplasma* Testing:**	Negative
BioSafety Level:	1

3. Materials Required, but Not Supplied

Note: Some part numbers differ outside of the continental United States. Please check with your local Invitrogen Technical Support.

Media/Reagents	Recommended Source	Part #
LiveBLAzer™ –FRET B/G Loading Kit, containing: LiveBLAzer™ -FRET B/G Substrate (CCF4-AM), DMSO, Solution B, and Solution C	Invitrogen	K1095 (0.2 mg) K1096 (1 mg) K1030 (5 mg)
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648-010
RPMI Medium 1640	Invitrogen	72400-047
Opti-MEM® I Reduced Serum Medium	Invitrogen	11058-021
DMSO	Fluka	41647
Dialyzed Fetal Bovine Serum (dFBS) (**DO NOT SUBSTITUTE!**)*	Invitrogen	26400-044
Nonessential amino acids (NEAA)	Invitrogen	11140-050
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140-122
Sodium Pyruvate	Invitrogen	11360-070
Recombinant Human GM-CSF	Invitrogen	PHC2015
JAK Inhibitor I	EMD	420009
Blasticidin (antibiotic)	Invitrogen	R210-01
Phosphate-buffered saline without calcium and magnesium [PBS(-)]	Invitrogen	14190-144

Consumables	Recommended Source	Part #
Black-wall, clear-bottom, 384-well assay plates (with low fluorescence background)**	Corning Life Sciences	3712
Compressed air	Various	—

Equipment	Recommended Source
Fluorescence plate reader with bottom-read capability***	Various
Filters, if required for plate reader (see **Section 5.3**)	Chroma Technology Corp.
Optional: Epifluorescence- or fluorescence-equipped microscope with appropriate filters	Various
Optional: Microplate centrifuge	Various

Notes: *The cell line has been grown in the presence of dialyzed FBS. Other forms of FBS may kill the cells, especially upon first thaw.

**Alternative assay plates (such as poly-D-lysine coated plates) may or may not work for this assay.

***If you do not have access to a fluorescence plate reader with bottom-read capability, contact our Technical Support at 1-760-603-7200, select option 3 and enter extension 40266. The assay conditions of this cell line have been fully validated with LiveBLAzer™ FRET B/G substrate and bottom-reading instruments. Other beta-lactamase substrates and top-reading method have not been tested with this cell line.

4. Cell Culture Conditions

4.1 Media Required

Component	Thaw Medium	Growth Medium	Assay Medium	Freezing Medium
RPMI Medium 1640	500 mL bottle	500 mL bottle	—	—
Opti-MEM® I Medium	—	—	500 mL bottle	—
FBS, dialyzed	50 mL	50 mL	2.5 mL	—
NEAA (10 mM)	5 mL	5 mL	5 mL	—
Sodium Pyruvate (100 mM)	5 mL	5 mL	5 mL	—
Penicillin (10,000 U/mL) / Streptomycin (10,000 µg/mL)	5 mL	5 mL	5 mL	—
Blasticidin	—	5 µg/mL	—	—
GM-CSF	2 ng/mL	2 ng/mL	—	—
Recovery™ Cell Culture Freezing Medium		—	—	100%

Note: We prepare our media by adding the listed components directly to the 500 mL medium bottle. GM-CSF is reconstituted to 10 ng/µL (7 µM) with PBS(-) + 0.1% BSA and stored in aliquots at –20°C for long-term storage (several months). GM-CSF aliquots are freeze/thaw sensitive, but once thawed they can be stored at 4°C for up to 1 week.

Note: Blasticidin and GM-CSF can be added directly to the cell culture flask to reach concentrations of 5 µg/mL and 2 ng/mL, respectively. Similar methods are suitable.

Note: Unless otherwise stated, have all media and solutions at least at room temperature (we recommend 37°C for optimal performance) before adding them to the cells.

4.2 Detailed Cell Handling Procedures

4.2.1 Special Considerations for working with this Cell Line

- This cell line is a clonal population isolated by Fluorescence Activated Cell Sorting (FACs) based on the functional response to GM-CSF. Assay performance can be expected to depend upon use of the specified media as responsive cells have been chosen based on these formulations.
- This cell line needs 2 ng/mL GM-CSF present in the growth medium to proliferate.
- Cells should double every 20–30 hours.
- Incubate cells in Assay Medium in the absence of GM-CSF for 16–20 hours prior to GM-CSF stimulation for the beta-lactamase assay.
- This cell line is blasticidin resistant.
- For additional information about the TF-1 cellular background please contact Technical Support at 1-760-603-7200, select option 3 and enter extension 40266.
- Cryopreserved irf1-bla TF-1 cells have been tested to be responsive to GM-CSF. For more information on cryopreserved cell provision services and using cryopreserved cells directly for the assay, please contact Technical Support.

4.2.2 Thawing Method

1. Place 40 mL of Thaw Medium (without Blasticidin) into a T225 flask.
2. Place the flask in a humidified 37°C/5% CO_2 incubator for 15 minutes to allow medium to equilibrate to the proper pH and temperature.
3. Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1–2 minutes. Do not submerge the vial in water.
4. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.

5. Transfer the vial contents into 10 mL of Thaw Medium (without Blasticidin) in a sterile 15-mL conical tube.
6. Centrifuge cells at 400 × *g* for 5 minutes.
7. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh Thaw Medium.
8. Count cells.
9. Transfer approximately 2×10^6 cells to the T225 tissue-culture flask containing pre-equilibrated 40 mL of Thaw Medium (final cell concentration ~50,000 cells/mL) and place flask in a humidified 37°C/5% CO_2 incubator.
10. Switch to passaging cells in Growth Medium with Blasticidin, once cells appear to be doubling every 20–30 hours.

4.2.3 Propagation Method

1. Cells should be passaged or fed at least twice a week.
2. Cells should be maintained at a density between 3×10^4 and 7×10^5 cells/mL. Do not allow cells to exceed 7×10^5 cells/mL as this may affect the cell viability and assay performance.
3. To passage cells, centrifuge the desired amount of cells suspension and resuspend with growth medium with fresh GM-CSF at a density of approximately 30,000 cells/mL.

4.2.4 Freezing Method

1. Harvest and count the cells, then spin cells down and resuspend in 4°C Recovery™ Cell Culture Freezing Medium at a desired cell density.
2. Dispense 1.0-mL aliquots into cryogenic vials.
3. Place in an insulated container for slow cooling and store overnight at –80°C.
4. Transfer to liquid nitrogen the next day for long term storage.

5. Assay Procedure

The following instructions outline the recommended procedure for monitoring JAK2/STAT5 signaling using LiveBLAzer™-FRET B/G Substrate as the readout.

Note:

- We recommend using 384-well, black-wall, clear-bottom assay plates with low fluorescence background.
- We recommend including cell-free control wells on the same plate as test wells for background subtraction. See **Section 6, Data Analysis**.
- Some solvents may affect assay performance. Assess the effect of a test compound solvent before screening. This cell line has been qualified for DMSO tolerance up to 1%. See validation packet at www.invitrogen.com/cellsensor for the assay performance of this cell line in the presence of various DMSO concentrations. The cell stimulation described below is carried out in the presence of 0.1% DMSO to simulate the effect that a test compound solvent might have on the assay. If you use other solvents and/or solvent concentrations, change the following assay conditions and optimize appropriately.

5.1 Quick Reference Guide

For more detailed protocol information, see **Section 5.2**.

Agonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Cell-free Wells	Test Compound Wells
Step 1 GM-CSF Starvation	Spin down appropriate number of cells grown in growth media and suspend in assay media lacking GM-CSF to a final cell concentration of 5×10^5 cells/mL. Incubate for 16-20 hours in tissue culture flask(s).			
Step 2 Harvest and Plate cells	32 µl cells in Assay Medium (50,000 cells/well)	32 µl cells in Assay Medium (50,000 cells/well)	32 µl Assay Medium (no cells)	32 µl cells in Assay Medium (50,000 cells/well)
Step 3 Add Agonist or Test Compounds	8 µl Assay Medium with 0.5% DMSO	8 µl 5X GM-CSF in Assay Medium with 0.5% DMSO	8 µl Assay Medium with 0.5% DMSO	8 µl 5X Test Compounds in 0.5% DMSO
Step 4 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 5 hours			
Step 5 Prepare 6X Substrate Mix	6 µl of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) Substrate + 60 µl of solution B, mix. Add 934 µl of Solution C, mix			
Step 6 Add Substrate Mixture	8 µl per well			
Step 7 Incubate	2.5 hours at room temperature in the dark			
Step 8 Detect activity	See **Section 5.3**			
Step 9 Analyze data	See **Section 6.**			

Antagonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Antagonist Control Wells	Cell-free Wells	Test Compound Wells
Step 1 GM-CSF Starvation	Spin down appropriate number of cells grown in growth media and suspend in assay media lacking GM-CSF to a final cell concentration of 5 x 10^5 cells/mL. Incubate for 16 - 20 hours in tissue culture flask(s).				
Step 2 Harvest and Plate cells	32 µl cells in Assay Medium (50,000 cells/well)	32 µl cells in Assay Medium (50,000 cells/well)	32 µl cells in Assay Medium (50,000 cells/well)	32 µl Assay Medium (no cells)	32 µl cells in Assay Medium (50,000 cells/well)
Step 3 Add Antagonist or Test Compounds	4 µl Assay Medium with 1% DMSO	4 µl Assay Medium with 1% DMSO	4 µl 10X Jak Inhibitor I in Assay Medium with 1% DMSO	4 µl Assay Medium with 1% DMSO	4 µl 10X Test Compounds in Assay Medium with 1% DMSO
Optional Step:	Incubate plate with Antagonist for 30 minutes before proceeding				
Step 4 Add Agonist	4 µl Assay Medium	4 µl 10X GM-CSF in Assay Medium	4 µl 10X GM-CSF in Assay Medium	4 µl 10X GM-CSF in Assay Medium	4 µl 10X GM-CSF in Assay Medium
Step 5 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 5 hours				
Step 6 Prepare 6X Substrate Mix	Add 6 µl of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) substrate + 60 µl of solution B, mix. Add 934 µl of Solution C, mix				
Step 7 Add Substrate Mixture	8 µl per well				
Step 8 Incubate	2.5 hours at room temperature in the dark				
Step 9 Detect activity	See **Section 5.3**				
Step 10 Analyze data	See **Section 6**				

5.2 Detailed Assay Protocol

Plate layouts and experimental outlines will vary. In screening mode, we recommend using at least three wells for each control: Unstimulated Control, Stimulated Control, and Cell-free Control.

5.2.1 Precautions and Special Considerations for Running this Assay

1. Work on a dust-free, clean surface. Always handle the 384-well, black-wall, clear-bottom assay plate by the sides; do not touch the clear bottom of the assay plate.
2. If pipetting manually, you may need to centrifuge the plate briefly at room temperature (30 seconds at 14 × *g*) after additions to ensure all assay components are on the bottom of the wells.
3. To obtain the proper assay window, cells must be washed free of GM-CSF and incubated for 16–20 hours in assay media.
4. Perform assay only if cells are doubling normally.

5.2.2 Prepare Cells for Assay

1. Three days prior to assay, cells are seeded in **Growth Media** at ~1 × 10^5 cells/mL
2. One day prior to assay, cells are harvested from growth media and counted. Cells should be at a cell density of ~4–7 × 10^5 cells/mL. **Do not exceed** 7 × 10^5 cells/mL.
3. Spin down the appropriate number of cells for assay at 400 × *g* for 5 minutes and remove media.
4. Wash cells by replacing with **Assay Media**, spin as before and remove media.
5. Resuspend cells in **Assay Media and** return to a tissue culture flask at a density of ~4 × 10^5 cells/mL.
6. Incubate cells in a 37°C/5%CO_2 incubator for 16–20 hours.

5.2.3 Plate Cells

1. Transfer the cells to a conical tube and count the number of live cells. Cells will not show typical growth rates (if any) during GM-CSF starvation.

2. Spin down the appropriate number of cells for assay 400 × g for 5 minutes and resuspend cells in Assay Medium at 1.6×10^6 cells/mL.
3. Add 32 µL per well of Assay Medium to the Cell-free control wells.
4. Add 32 µL per well of the cell suspension to the Test Compound wells, the Unstimulated Control wells, and Stimulated Control wells.
5. Incubate the plates in a 37°C/5% CO_2 incubator while preparing the Compound and GM-CSF control solutions.
6. Proceed to **Section 5.2.4** for an Agonist assay or **Section 5.2.5** for an Antagonist assay.

5.2.4 Agonist Assay Plate Setup

Note: This section provides directions for performing an Agonist assay. See **Section 5.2.5** for directions for performing an Antagonist assay.

Note: The positive agonist controls are run at the concentration of GM-CSF that gives the maximum stimulation (top of the dose response curve). We recommend running a dose response curve to determine the optimal concentration for your GM-CSF solution. See **Section 6.3** for a representative curve.

1. Prepare a stock solution of 0.5% DMSO in Assay Medium.
2. Prepare a 5X stock of Test Compounds in Assay Medium with 0.5% DMSO (or if test compound is dissolved in DMSO, make sure the DMSO concentration for the 5X solution is 0.5%).
3. GM-CSF is reconstituted to 10 ng/µL (7 µM) with PBS(-) + 0.1% BSA and stored in aliquots at -20°C for long-term storage (several months). GM-CSF aliquots are freeze/thaw sensitive, but once thawed it can also be stored at 4°C for up to 1 week.
4. Prepare a 5X stock of GM-CSF in Assay Medium containing 0.5% DMSO. For maximum stimulation, use ~4 ng/mL (0.2 nM) final concentration (5X = 1 µM). We recommend running a dose response curve to determine the EC100 for your GM-CSF solution. See **Section 6.3** for a representative curve.
5. Add 8 µl of the stock solution of 0.5% DMSO in Assay Medium to the Unstimulated Control and Cell-free Control wells.
6. Add 8 µl of the 5X stock solution of GM-CSF to the Stimulated Control wells.
7. Add 8 µl of the 5X stock of Test Compounds to the Test Compound wells.
8. Incubate the Agonist assay plate in a humidified 37°C/5% CO_2 incubator for 5 hours. Then proceed to **Section 5.2.6** for Substrate Loading and Incubation.

5.2.5 Antagonist Assay Plate Setup

Note: This section provides directions for performing an Antagonist assay. See **Section 5.2.4** for directions for performing an Agonist assay.

1. Prepare a stock solution of 1% DMSO in Assay Medium.
2. Prepare a 10X stock of Test Compounds in Assay Medium with 1% DMSO. (Or if test compound is dissolved in DMSO, prepare a 10X stock of Test Compounds in Assay Medium and make sure the DMSO concentration for the 10X solution is 1.0%).
3. GM-CSF is reconstituted to 10 ng/µL (7 µM) with PBS(-) + 0.1% BSA and stored in aliquots at –20°C for long-term storage (several months). GM-CSF aliquots are freeze/thaw sensitive, but once thawed it can also be stored at 4°C for up to 1 week.
4. Prepare a 10X stock of GM-CSF in Assay Medium at an EC_{80} concentration. For an example EC_{80} concentration, use ~1.5 ng/mL (0.1 nM) final concentration (10X =1 nM). We recommend running a dose response curve to determine the EC_{80} for your GM-CSF solution. See **Section 6.3** for a representative curve.
5. Prepare a 10X stock of Jak Inhibitor I (control antagonist compound) in Assay Medium with 1.0% DMSO. For maximum inhibition, use 8.8 µM final concentration (10X = 88 µM). We recommend running a dose response curve to determine the optimal inhibition concentration for the Antagonist solution.
6. Add 4 µl of the 10X stock of Test Compounds to the Test Compound wells.
7. Add 4 µl of the stock solution of 1.0% DMSO to the Stimulated Control wells, the Unstimulated Control wells, and the Cell-free Control wells.

8. Add 4 µl of the 10X stock of Jak Inhibitor I to the Antagonist Control wells.
9. If desired, incubate the Test Compounds with the cells in a humidified 37°C/5% CO_2 incubator before proceeding. Typically, a 30-minute incubation is sufficient.
10. Add 4 µl of the 10X EC_{80} stock solution of GM-CSF prepared in step 4 to the Test Compound wells, the Stimulated Control wells, and the Antagonist Control wells.
11. Add 4 µl of Assay Medium to the Unstimulated Control and Cell-free Control wells.
12. Incubate the Antagonist assay plate in a humidified 37°C/5% CO_2 incubator for 5 hours. Then proceed to **Section 5.2.6** for Substrate Loading and Incubation.

5.2.6 Substrate Loading and Incubation

This protocol is designed for loading cells with LiveBLAzer™-FRET B/G Substrate (CCF4-AM). If alternative substrates are used, please follow the loading protocol provided with the substrate.

Preparation of 6X LiveBLAzer™-FRET B/G Substrate (CCF4-AM) mixture and cell loading should be done quickly in the absence of direct strong lighting.

1. Prepare Solution A: 1 mM LiveBLAzer™-FRET B/G Substrate (CCF4-AM, MW = 1096) stock solution. Add anhydrous DMSO provided directly to the vial of lyophilized CCF4-AM, using 182 µL of DMSO for every 200 µg of CCF4-AM. Mix well. Store the aliquots of the stock solution at –20°C until use.
2. Prepare 6X LiveBLAzer™-FRET B/G (CCF4-AM) Substrate Mixture:

   2.1 Add 6 µL of Solution A to 60 µL of Solution B and vortex.

   2.2 Add 934 µL of Solution C to the combined solutions from above step with vortexing.

   Note: If more than 1 mL 6X Substrate Mixture is needed, scale up the amount of each solution proportionally.
3. Remove assay plate from the humidified 37°C/5% CO_2 incubator.
4. Add 8 µL of 6X Substrate Mixture from **Step 2** to each well.
5. Cover the plate to protect it from light and evaporation.
6. Incubate at room temperature for 2.5 hours.

5.3 Detection

All measurements using LiveBLAzer™-FRET B/G Substrate should be made at room temperature from the bottom of the wells. Before reading the plate, remove dust from the bottom with compressed air.

5.3.1 Microplate Readers and Optical Requirements

- Most fluorescence microplate readers (filter or monochromator) with bottom-reading capabilities are suitable for detection. For a current list of compatible microplate readers, contact Drug Discovery Technical Support at 1-800-955-6280, select option 3, and enter extension 40266.
- Recommended filters (or those with similar spectral specifications) for fluorescence microplate readers are listed below, and are also available from Chroma Technologies (800-824-7662, www.chroma.com)

  Excitation filter: 405/20 nm (Chroma part# HQ405/20x)

  Emission filter: 460/40 nm (Chroma part# HQ460/40m)

  Emission filter: 530/30 nm (Chroma part# HQ530/30m)
- Recommended dichroic mirrors: 380 nm, 400 nm, and 425 nm cutoff mirrors have been successfully used, and general 50/50 mirrors may also be suitable.
- Refer to ToxBLAzer™ protocol for specific instrumentation and filter recommendations.

5.3.2 Reading an Assay Plate

Set the fluorescence plate reader to bottom-read mode, and establish a dual-emission wavelength measurement protocol within the instrument software (i.e., measurement 1 at 405 nm excitation and 460 nm emission; measurement 2 at 405 nm excitation and 530 nm emission). Some instrument settings may also require optimization (e.g., gain, plate height, flash number, integration time, etc.).

For specific microplate reader setup information, contact Drug Discovery Technical Support at 1-800-955-6280, select option 3 and enter extension 40266.

6. Data Analysis

6.1 Background Subtraction and Blue/Green Ratio Calculation

We recommend that you subtract the background for both emission channels (460 nm and 530 nm).

1. Use the assay plate layout to identify the location of the Cell-free wells. These control wells are used for background subtraction.
2. Determine the average emission from the Cell-free wells at both 460 nm (Average Blue Background) and 530 nm (Average Green Background).
3. Subtract the Average Blue Background (data collected at 460 nm) from all of the blue emission data.

Note: Background-corrected values should not be near zero.

4. Subtract the Average Green background (data collected at 530 nm) from all of the green emission data.

Note: Background-corrected values should not be near zero.

5. Calculate the Blue/Green Emission Ratio for each well by dividing the background-subtracted blue emission values by the background-subtracted green emission values.

Note: You may also calculate the response ratio to determine the assay window. The response ratio is calculated as the Blue/Green Emission Ratio of the GM-CSF –Stimulated wells divided by the Blue/Green Emission Ratio of the unstimulated wells. Generally, a response ratio of >3 has been shown to yield a $Z' \geq 0.5$.

6.2 Visual Observation of Intracellular Beta-lactamase Activity Using LiveBLAzer™-FRET B/G Substrate (CCF4-AM)

***Note*:** Microscopic visualization of cells will cause photobleaching. Always read the assay plate in the fluorescence plate reader before performing microscopic visualization.

An inverted microscope equipped for epifluorescence and either a xenon or mercury excitation lamp are typically required to view the LiveBLAzer™-FRET B/G Substrate (CCF4-AM) signal in cells. To visually inspect the cells, you will need a long-pass filter passing blue and green fluorescence light so that your eye can visually identify whether the cells are fluorescing green or blue.

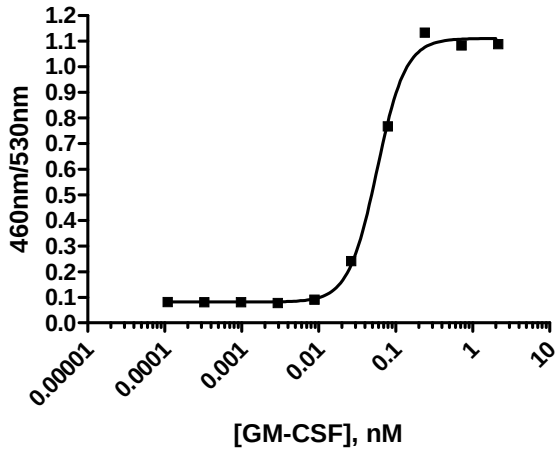
Recommended filter sets for observing beta-lactamase activity are described below and are available from Chroma Technologies (800-824-7662, www.chroma.com).

Chroma Set # 41031

Excitation filter: HQ405/20x (405 ± 10 nm)
Dichroic mirror: 425 DCXR
Emission filter: HQ435LP (435 long-pass)

Filter sizes vary for specific microscopes and need to be specified when the filters are ordered. For epifluorescence microscopes, a long-pass dichroic mirror is needed to separate excitation and emission light and should be matched to the excitation filter (to maximally block the excitation light around 405 nm, yet allow good transmission of the emitted light).

6.3 Representative Data



EC_{50}	0.057 nM
EC_{80}	0.1 nM
EC_{100}	0.2 nM
Z′ at EC_{100}	0.89

Figure 1. Dose response of irf1-*bla* TF-1 cells to hGM-CSF. CellSensor® irf1-*bla* TF-1 cells were GM-CSF starved and then treated with agonist GM-CSF over the indicated concentration range in a 384-well format. Cells were incubated for 5 hours with agonist and 0.1% DMSO and then combined with LiveBLAzer™-FRET B/G Substrate (CCF4-AM) for 2.5 hours. A Tecan Safire2® fluorescent plate reader was used to measure fluorescence emission values at 460 nm and 530 nm, and the 460/530 ratios were plotted against the concentration of the agonist.

7. References

1. Zlokarnik, G., *et al,* **Quantitation of Transcription and Clonal Selection of Single Living Cells with Beta-Lactamase as Reporter**, (1998) *Science*; 279: p84-88.

2. Kunapuli P., Ransom R., Murphy K., Pettibone D., Kerby J., Grimwood S., Zuck P., Hodder P., Lacson R., Hoffman I., Inglese J., Strulovici B., **Development of an Intact Cell Reporter Gene Beta-lactamase Assay for G Protein-coupled Receptors, (**2003) *Analytical Biochem.;* **314:** p16-29.

3. Xing, H., Pollok, B., *et al*, **A Fluorescent Reporter Assay For The Detection of Ligands Acting Through G1 Protein-coupled Receptors,** (2000) *J. Receptor & Signal Transduction Research*; **20**: p189-210.

4. Peekhaus, N. *et al,* A **Beta-Lactamase-Dependent Gal4-Estrogen Receptor Transactivation Assay for the Ultra-High Throughput Screening of Estrogen Receptor Agonists in a 3,456-Well Format**, (2003) *Assay and Drug Dev Tech*; **1**: p789-800.

5. Chin**, J., *et al*, Miniaturization of Cell-Based Beta-Lactamase-Dependent FRET Assays to Ultra-High Throughput Formats to Identify Agonists of Human Liver X Receptors**, (2003) *Assay and Drug Dev. Tech.*; **1**: p777-787.

6. Whitney M., Rockenstein E., Cantin G., Knapp T., Zlokarnik G., Sanders P., Durick K., Craig F.F., Negulescu P.A., **A Genome-wide Functional Assay of Signal Transduction in Living Mammalian Cells,** (1998) *Nat. Biotechnol.*; **16**: p1329-1333.

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Use of Genetically Modified Organisms (GMO)

Information for European Customers: The CellSensor® irf1-*bla* TF-1 cell line(s) are genetically modified with the plasmid pLenti-*bsd*/if1-*bla*. As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.