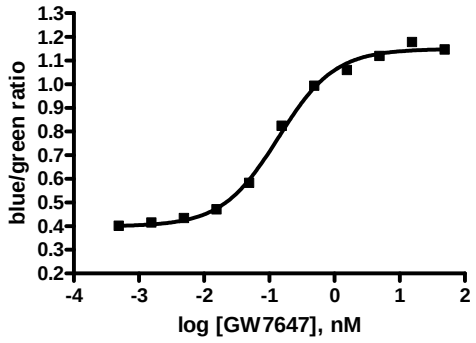
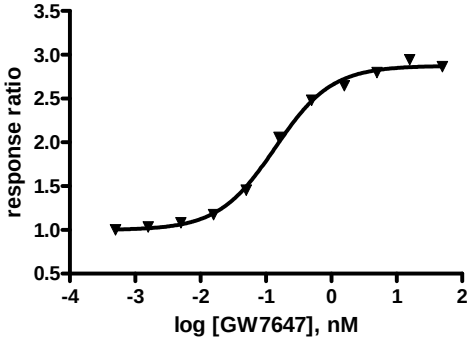
Table of Contents

1. Description

GeneBLAzer® PPAR alpha UAS-bla HEK293T cells contain a PPAR alpha ligand-binding domain/Gal4 DNA binding domain chimera transiently transduced (via BacMam virus) into the CellSensor® UAS-bla HEK293T cell line. CellSensor® UAS-bla HEK293T contains a beta-lactamase reporter gene under control of a UAS response element stably integrated into HEK293T cells. While this assay has a lower assay window and Z′ than other GeneBLAzer® assays due to the biology of the receptor, it is still suitable for profiling. This assay provides accurate pharmacology and provides robust EC_{50} and IC_{50} values.





EC_{50}	0.14 nM
Z′-factor at EC_{100}	0.6

Dose response of PPAR alpha UAS-*bla* HEK293T cells to GW7647. PPAR alpha-UAS-*bla* HEK293T cells were plated at 20,000 cells/well in a 384 well poly-D-lysine plate and stimulated with GW7647 in the presence of 0.1% DMSO for 18 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 µm final concentration of substrate) for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader in bottom-read mode. Curves were fit using a sigmoidal dose-response equation (variable slope) in GraphPad™ Prism® 4.0. The graphs on the right and left are data plotted differently. The values plotted in the graph on the left are background-subtracted blue/green ratios. The values plotted in the graph on the right are background-subtracted response ratios. (Response ratio is the blue/green ratio of the stimulated cells divided by the blue/green ratio of the unstimulated cells and is a measure of the assay window). There is no effect on the Z′ value or calculated EC_{50}.

2. Overview of GeneBLAzer® Beta-Lactamase Reporter Technology

GeneBLAzer® Beta-lactamase Reporter Technology provides a highly accurate, sensitive, and easy-to-use method of monitoring the cellular response 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 dual-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 in high-throughput screening (HTS) campaigns for a range of target classes, including G-protein coupled receptors (GPCRs) (2, 3), nuclear receptors (4-6) and kinase signaling pathways (7).

3. Materials Supplied

Product:	**GeneBLAzer® PPAR alpha UAS-*bla* HEK293T Assay Kit** Each system contains sufficient transduced cells and substrate to assay one 384-well plate. Includes: • PPAR alpha UAS-*bla* HEK293T cells (K1875A) • LiveBLAzer™-FRET B/G Loading Kit, 70 µg
Size:	1 plate
Catalog no.	K1875 (cell line part no. K1875A)
Shipping:	Dry ice
Storage of Cells:	**Short Term:** –80°C **Long Term:** Liquid nitrogen **Immediately upon receipt, cells should be stored in liquid nitrogen or thawed for immediate use. Cells stored at –80°C can quickly lose viability.**
Growth Properties of Cells:	Adherent
Cell Phenotype:	Epithelial
Selection Marker(s) for cells:	Not applicable, this cell line cannot be cultured
***Mycoplasma* Testing:**	Negative
BioSafety Level:	2

4. Materials Required

Use the tables below to determine the additional media and reagents required for use:

Media/Reagents	Recommended Source	Part #
DMSO	Fluka	41647
Phenol Red-free DMEM	Invitrogen	21063-029
Bovine Serum Albumin (BSA) from Sigma (catalog no. A8806). **Do not substitute vendor or part number.**	Sigma	A8806
GW7647	Calbiochem	370698
Dulbecco's Phosphate Buffered Saline (dPBS) without Ca^{2+} and Mg^{2+}	Invitrogen	14190-144

Consumables	Recommended Source	Part #
Black-wall, clear-bottom, Poly-D-Lysine coated 384 well assay plates	Corning	3664
Compressed air	Various	—

Equipment	Recommended Source
Fluorescence plate reader with bottom-read capabilities	Various
Filters if required for plate reader (see **Section 7.4.1**)	Chroma Technologies
Optional: Epifluorescence- or fluorescence-equipped microscope, with appropriate filters	Various
Optional: Microplate centrifuge	Various

5. Media Requirements

To prepare Assay Medium, add BSA (Sigma catalog no. A8806) to Phenol Red-free DMEM at a final concentration of 0.01%. The BSA from Sigma is supplied as a powder and needs to be dissolved in sterile dPBS to make a 10% solution (w/v) before adding to the DMEM.

Example: Dissolve 1 g of BSA powder in 10 mL of sterile dPBS to make a 10% solution. Add 500 µL of that solution to 500 mL of Phenol Red-free DMEM for a final concentration of 0.01% BSA. Ensure that the BSA is completely dissolved prior to addition.

Note: Make **NO MEDIA or BSA SUBSTITUTIONS**, as this cell line has been specifically validated for optimal assay performance with this media. Use of an alternate BSA vendor or part number will likely result in **significantly** decreased assay response.

Component	Assay Medium
Phenol Red-free DMEM	500 mL
BSA (from Sigma, catalog no. A8806) dissolved in sterile dPBS—10% solution	500 µL (0.01% final concentration)

Note: We prepare our media by adding the listed components directly to the medium bottle. For example, add 500 µL of the 10% BSA solution to the 500 mL bottle of media. Similar methods are suitable.

Note: Unless otherwise stated, all media and solutions should be at room temperature or warmer (we recommend 37°C for optimal performance) before adding to cells.

6. Thawing Cells

Refer to **Section 5, Media Requirements**, for instructions on preparing Assay Medium.

Note: Once cells are thawed per the instructions below, cells must be counted and the density adjusted to the appropriate level as specified in **Section 7, Assay Procedure**, prior to analysis.

1. Rapidly thaw the vial of cells by placing at 37°C in a water bath with gentle agitation for 1–2 minutes. Do not submerge vial in water.
2. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
3. Transfer the vial contents drop-wise into 10 mL of Assay Medium (prepared as specified in Section 5) in a sterile 15-mL conical tube.
4. Centrifuge cells at 200 × *g* for 5 minutes.
5. Aspirate supernatant and resuspend the cell pellet in 5 mL dPBS.
6. Centrifuge cells at 200 × *g* for 5 minutes.
7. Aspirate supernatant and resuspend the cell pellet in 1 mL fresh Assay Medium.
8. Count the cells.
9. Proceed to **Section 7, Assay Procedure**. Adjust the cell density with Assay Medium to the appropriate cell density as specified in Section 7.

7. Assay Procedure

The following instructions outline the recommended procedure for determining activity of compounds as modulators of PPAR alpha using LiveBLAzer™-FRET B/G Substrate as the readout. If using ToxBLAzer™ DualScreen, follow the loading protocol provided with that product.

7.1 Quick Assay Reference Guides

For a more detailed assay protocol, see **Section 7.2**.

Agonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Cell-free Wells	Test Compound Wells
Step 1 Plate cells	32 µL cells in Assay Medium (20,000 cells/well)	32 µL cells in Assay Medium (20,000 cells/well)	32 µL Assay Medium (no cells)	32 µL cells in Assay Medium 20,000 cells/well)
Step 2 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for ~3 hours			
Step 3 Add Agonist or Test Compounds	8 µL Assay Medium with 0.5% DMSO	8 µL 5X GW7647 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 16–19 hours			
Step 5 Prepare 6X Substrate Mix	Add 6 µL of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) Substrate with 60 µL of solution B, mix. Add 934 µL of Solution C, mix. Solution D is not required.			
Step 6 Add Substrate Mixture	8 µL per well for all wells			
Step 7 Incubate mixture	2 hours at room temperature in the dark			
Step 8 Detect activity	See **Section 7.3**			
Step 9 Analyze data	See **Section 8**			

Antagonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Antagonist Control Wells	Cell-free Wells	Test Compound Wells
Step 1 Plate cells	32 µL cells in Assay Medium (20,000 cells/well)	32 µL cells in Assay Medium (20,000 cells/well)	32 µL cells in Assay Medium (20,000 cells/well)	32 µL Assay Medium (no cells)	32 µL cells in Assay Medium (20,000 cells/well)
Step 2 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for ~3 hours				
Step 3 Add Antagonist or Test Compounds	4 µL Assay Medium with 0.5% DMSO	4 µL Assay Medium with 0.5% DMSO	4 µL 10X antagonist in Assay Medium with 0.5% DMSO	4 µL Assay Medium with 0.5% DMSO	4 µL 10X Test Compounds in Assay Medium with 0.5% DMSO
Optional Step:	Incubate plate with Antagonist for 30 minutes before proceeding				
Step 4 Add Agonist	4 µL Assay Medium with 0.5% DMSO	4 µL 10X GW7647 in Assay Medium with 0.5% DMSO	4 µL 10X GW7647 in Assay Medium with 0.5% DMSO	4 µL 10X GW7647 in Assay Medium with 0.5% DMSO	4 µL 10X GW7647 in Assay Medium with 0.5% DMSO
Step 5 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 16–19 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. Solution D is not required.				
Step 7 Add Substrate Mixture	8 µL per well for all wells				
Step 8 Incubate mixture	2 hours at room temperature in the dark				
Step 9 Detect activity	See **Section 7.4**				
Step 10 Analyze data	See **Section 8**				

7.2 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.

Note: Some solvents may affect assay performance. Assess the effects of solvent before screening. The cell stimulation procedure described below is carried out in the presence of 0.1% DMSO to simulate the effect that a Test Compound's solvent might have on the assay. If you use other solvents and/or solvent concentrations, optimize the following assay conditions appropriately.

Note: GW7647 is provided as a powder. We recommend using DMSO to reconstitute to a 10 mM stock solution. Aliquots can be stored at -20°C for several months.

7.2.1 Precautions

- 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.
- If pipetting manually, you may need to centrifuge the plate briefly at room temperature (for 1 minute at 14 × *g*) after additions to ensure all assay components are on the bottom of the wells.

7.2.2 Plating Cells

1. Thaw cells as described in Section 6 and resuspend in Assay Medium to a density of 6.25×10^5 cells/mL.
2. Add 32 µL per well of the Assay Medium to the Cell-free Control wells. Add 32 µL per well of the cell suspension to the Test Compound wells, the Unstimulated Control wells, and Stimulated Control wells.

Important: Incubate the assay plate in a humidified 37°C/5% CO_2 incubator for ~3 hours to allow the cells to attach.

Proceed to **Section 7.2.3** for an Agonist assay or **Section 7.2.4** for an Antagonist assay.

7.2.3 Agonist Assay Plate Setup

Note: This subsection provides directions for performing an Agonist assay. See **Section 7.2.4** for directions for performing an Antagonist assay.

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 the test compounds are dissolved in DMSO, make sure the DMSO concentration for the 5X solution is 0.5%). If performing a dilution series, we recommend performing the serial dilutions in 100% DMSO at 1000X compound concentration. Then use Assay Medium to dilute compounds to 5X and 0.5% DMSO. Performing the serial dilutions in 100% DMSO stabilizes EC_{50} values by minimizing problems with compounds coming out of solution while preparing the dilutions.
3. Prepare a 5X stock of GW7647 in Assay Medium with 0.5% DMSO (or if GW7647 is dissolved in DMSO, make sure the DMSO concentration for the 5X solution is 0.5%). We recommend running a dose response curve to determine the optimal concentration for the GW7647 solution.
4. Add 8 µL of the stock solution of 0.5% DMSO in Assay Medium to the Unstimulated Control and Cell-free Control wells.
5. Add 8 µL of the 5X stock solution of GW7647 to the Stimulated Control wells.
6. Add 8 µL of the 5X stock of Test Compounds to the Test Compound wells.
7. Incubate the Agonist assay plate in a humidified 37°C/5% CO_2 incubator for ~16-19 hours. Then proceed to **Section 7.3** for Substrate Loading and Incubation.

7.2.4 Antagonist Assay Plate Setup

Note: This subsection provides directions for performing an Antagonist assay. See **Section 7.2.3** for directions for performing an Agonist assay.

1. Prepare a stock solution of 0.5% DMSO in Assay Medium.
2. Prepare a 10X stock of Test Compounds in Assay Medium with 0.5% DMSO (or if the test compounds are dissolved in DMSO, make sure the DMSO concentration for the 10X solution is 0.5%). If performing a dilution series, we recommend performing the serial dilutions in 100% DMSO at 2000X compound concentration. Then use Assay Medium to dilute compounds to 10X and 0.5% DMSO. Performing the serial dilutions in 100% DMSO stabilizes IC_{50} values by minimizing problems with compounds coming out of solution while preparing the dilutions.
3. Prepare a 10X stock of GW7647 in Assay Medium with 0.5% DMSO (or if GW7647 is dissolved in DMSO, make sure the DMSO concentration for the 10X solution is 0.5%). It is important to first perform a dose

response curve to determine the optimal agonist concentration at which to perform the antagonist testing. For antagonist assays, we recommend stimulating cells with an agonist concentration in the ~EC_{80} range.

4. Prepare a 10X stock of antagonist in Assay Medium with 0.5% DMSO (or if the antagonist is dissolved in DMSO, make sure the DMSO concentration for the 10X solution is 0.5%). We recommend running a dose response curve to determine the optimal inhibition concentration for the Antagonist solution.
5. Add 4 µL of the 10X stock of Test Compounds to the Test Compound wells.
6. Add 4 µL of the stock solution of 0.5% DMSO in Assay Medium to the Stimulated Control wells, the Unstimulated Control wells, and the Cell-free Control wells.
7. Add 4 µL of the 10X stock of antagonist in Assay Medium with 0.5% DMSO to the Antagonist Control wells.
8. 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.
9. Add 4 µL of the 10X stock solution of GW7647 with 0.5% DMSO to the Test Compound wells, the Stimulated Control wells, and the Antagonist Control wells.
10. Add 4 µL of Assay Medium with 0.5% DMSO to the Unstimulated Control and Cell-free Control wells.
11. Incubate the Antagonist assay plate in a humidified 37°C/5% CO_2 incubator for ~16-19 hours. Then proceed to **Section 7.3** for Substrate Loading and Incubation.

7.3 Substrate Preparation, Loading, and Incubation

This protocol is designed for loading cells with LiveBLAzer™-FRET B/G Substrate Mixture (CCF4-AM) Substrate Mixture. If you are using ToxBLAzer™ DualScreen, follow the loading protocol provided with that substrate.

Prepare LiveBLAzer™-FRET B/G Substrate Mixture (CCF4-AM) Substrate Mixture and load cells in the absence of direct strong lighting. Turn off the light in the hood.

1. Prepare Solution A: 1 mM LiveBLAzer™-FRET B/G Substrate (CCF4-AM) Substrate Mixture in dry DMSO. Store the aliquots of the stock solution at –20ºC until use. The molecular weight of the LiveBLAzer™-FRET B/G Substrate (CCF4-AM) is 1096 g/mol.

   For example: Add 64 µL of the supplied dry DMSO to the 70 µg of substrate for a 1 mM solution.

2. Prepare 6X Loading Solution:

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

   b. Add 934 µL of Solution C to the above solution and vortex.

   c. Solution D is not used.

   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.

   Note: Handle the plate gently and do not touch the bottom.

4. Add 8 µL of the 6X Substrate Mixture to each well.
5. Cover the plate to protect it from light and evaporation.
6. Incubate at **room temperature** for 2 hours.

7.4 Detection

Make measurements at room temperature from the bottom of the wells, preferably in 384-well, black-wall, clear-bottom assay plates with low fluorescence background. Before reading the plate, remove dust from the bottom with compressed air.

7.4.1 Instrumentation, Filters, and Plates

- Fluorescence plate reader with bottom reading capabilities.
- Recommended filters for fluorescence plate reader:
  Excitation filter: 409/20 nm
  Emission filter: 460/40 nm
  Emission filter: 530/30 nm

7.4.2 Reading an Assay Plate

1. Set the fluorescence plate reader to bottom-read mode with optimal gain and 5 reads.
2. Allow the lamp in the fluorescence plate reader to warm up for at least 10 minutes before making measurements.
3. Use the following filter selections:

	Scan 1	Scan 2
Purpose:	Measure fluorescence in the Blue channel	Measure FRET signal in the Green channel
Excitation filter:	409/20 nm	409/20 nm
Emission filter:	460/40 nm	530/30 nm

8. Data Analysis

8.1 Background Subtraction and 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 Control wells. These Control wells are used for background subtraction.
2. Determine the average emission from the Cell-free Control wells at both 460 nm (Average Blue Background) and 530 nm (Average Green Background).
3. Subtract the Average Blue background from all of the Blue emission data.
4. Subtract the Average Green background from all of the Green emission data.
5. Calculate the Blue/Green Emission Ratio for each well, by dividing the background-subtracted Blue emission value of each well by the background-subtracted Green emission value of each well.

Note: You may also calculate response ratio to know your assay window. The response ratio is calculated as the Blue/Green Emission Ratio of the GW7647 –Stimulated wells divided by the Blue/Green Emission Ratio of the unstimulated wells.

8.2 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 with either a xenon or mercury excitation lamp may be used 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)
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).

9. 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. Qureshi, S., *et al,* **A One-Arm Homologous Recombination Approach for Developing Nuclear Receptor Assays in Somatic Cells,** (2003) *Assay and Drug Dev. Tech;* **1**: p755-766.
5. 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.
6. 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.
7. Whitney M, Rockenstein E, Cantin G, Knapp T, Zlokarnik G, Sanders P, Durick K, Craig FF, Negulescu PA., **A Genome-wide Functional Assay of Signal Transduction in Living Mammalian Cells,** (1998) *Nat. Biotechnol.*;**16**: p1329-1333.

10. Purchaser Notification

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Use of Genetically Modified Organisms (GMO)

Information for European Customers The PPAR alpha UAS-*bla* HEK293T cell line is genetically modified with the plasmids pLenti5/UAS-BLA-Bsd and pFB CMV Gal4-PPARalpha LBD. 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.