GeneBLAzer® Gqo5 -NFAT-*bla* CHO-K1 Cell-based Assay

invitrogen™

Cat. no. K1220 | **Shipping: Dry Ice** | **Storage: Liquid Nitrogen**

Protocol part no. K1536.pps | Rev. date: 28 October 2010

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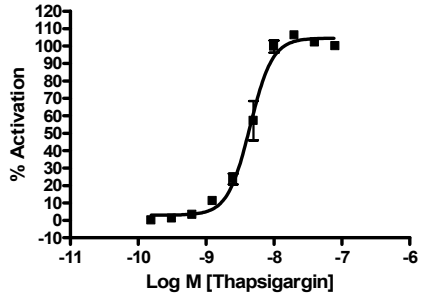
1. Description

GeneBLAzer® Gqo5-NFAT-*bla* CHO-K1 cells contain the chimeric G protein Gqo5 stably integrated into the CellSensor® NFAT-bla CHO-K1 cell line. CellSensor® NFAT-bla CHO-K1 contains a beta-lactamase reporter gene under control of a NFAT response element stably integrated into CHO-K1 cells.

GeneBLAzer® Gqo5-NFAT-*bla* CHO-K1 cells are designed for the construction of Gi/o-coupled GPCR cell-based assays. Using Lipofectamine™ 2000, you transfect a plasmid containing your Gi/o GPCR of interest into this cell line, and the chimeric Gqo5 protein couples the Gi/o GPCR to the NFAT-*bla* reporter construct. The cell line has also been functionally validated for Z′-Factor and EC_{50} concentrations of Thapsigargin.



Dose response of Gqo5-NFAT-*bla* CHO-K1 cells to Thapsigargin.

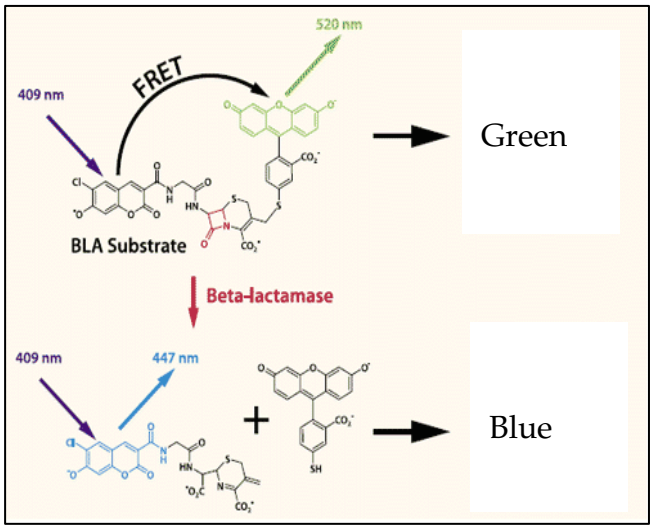
EC_{50}	**4.4 nM**
Z′-factor at EC_{100}	**0.79**

2. Overview of GeneBLAzer® GPCR Cell-based Assays

The GeneBLAzer® GPCR Cell-based Assay provides a highly accurate, sensitive, and easy-to-use method of monitoring cellular response to drug candidates or other stimuli (1). The core of the GeneBLAzer® assay technology is a beta-lactamase (*bla*) 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® GPCR assay 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).

The GeneBLAzer® assay technology uses a mammalian-optimized *bla* reporter gene combined with a FRET-enabled substrate to provide reliable and sensitive detection in cells (1) (Figure 1). Cells are loaded with an engineered fluorescent substrate containing two fluorophores: coumarin and fluorescein. In the absence of *bla* expression, the substrate molecule remains intact. In this state, excitation of the coumarin results in fluorescence resonance energy transfer to the fluorescein moiety and emission of green fluorescent light. However, in the presence of *bla* expression, the substrate is cleaved separating the fluorophores and disrupting energy transfer. Excitation of the coumarin in the presence of *bla* enzyme activity results in a blue fluorescence signal.

Figure 1.



Fluorescent detection of beta-lactamase reporter gene response using a FRET-enabled substrate. After substrate loading, in the absence of beta-lactamase expression, cells appear green fluorescent. In the presence of beta-lactamase expression, the substrate is cleaved and cells appear blue fluorescent.

3. Materials Supplied

Product:	Name	Size	Catalog #
	GeneBLAzer® Gqo5-NFAT-*bla* CHO-K1 cells Includes: • GeneBLAzer® Gqo5-NFAT-*bla* CHO-K1 cells (K1220) • Protocol • Certificate of Analysis	1 tube	K1220
Shipping Condition:	Dry ice		
Storage Condition of Cells:	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.**		
Growth Properties of Cells:	Adherent		
Cell Phenotype:	Epithelial		
Selection Marker(s) for Cells:	Zeocin™ 100 µg/ml; Geneticin® 500 µg/ml;		
***Mycoplasma* Testing:**	Negative		
BioSafety Level:	1		

4. Materials Required

Media/Reagents	Recommended Source	Part #
LiveBLAzer™-FRET B/G Loading Kit: LiveBLAzer™-FRET B/G Substrate (CCF4-AM) DMSO for Solution A Solution B Solution C	Invitrogen	K1427 (70 µg) K1095 (200 µg) K1096 (1 mg) K1030 (5 mg)
Solution D	Invitrogen	K1156 (1 ml) K1157 (25 ml)
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648-010
DMEM (high-glucose), with GlutaMAX™	Invitrogen	10569-010
DMSO	Fluka	41647
Fetal bovine serum (FBS), dialyzed, (**DO NOT SUBSTITUTE!**)	Invitrogen	26400-036
Non-essential amino acids (NEAA)	Invitrogen	11140-050
Penicillin/Streptomycin (antibiotics)	Invitrogen	15140-122
Phosphate-buffered saline without calcium and magnesium [PBS(-)]	Invitrogen	14190-136
HEPES (1 M, pH 7.3)	Invitrogen	15630-080
0.05% Trypsin/EDTA	Invitrogen	25300-054
Thapsigargin	Calbiochem	586005
Zeocin™	Invitrogen	R250-01
Geneticin®	Invitrogen	10131-027
Lipofectamine™ 2000	Invitrogen	11668-019
pcDNA™6.2-hygro-DEST Vector (or other vector that does not contain a Zeocin™ or Geneticin® resistance gene)	Invitrogen	K1233
Opti-MEM® I Medium	Invitrogen	11058-021

Consumables	Recommended Source	Part #
Black-wall, clear-bottom, 96 well assay plates (with low fluorescence background)	Corning	3603
Black-wall, clear-bottom, 384-well assay plates (with low fluorescence background)	Corning	3712
Compressed air	Various	---
Equipment	**Recommended Source**	
Fluorescence plate reader with bottom-read capabilities	Various	
Filters if required for plate reader (see **Section 9**)	Chroma Technologies	

4.1 Optional Equipment and Materials

- Epifluorescence- or fluorescence-equipped microscope, with appropriate filters
- Microplate centrifuge

5. Detailed Cell Handling Procedures

Note: Refer to **Section 6, Media Requirements** for specific media recip*es.*

5.1 Thawing Method

Note: Cells are shipped on dry ice and as such may require a short period of time prior to full recovery and normal growth.

1. Place 14 ml of Thawing Medium into a T75 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 rapidly thaw by placing at 37°C in a water bath with gentle agitation for 1–2 minutes. Do not submerge 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 drop-wise into 10 ml of Thawing Medium in a sterile 15-ml conical tube.
6. Centrifuge cells at 200 × *g* for 5 minutes.
7. Aspirate supernatant and resuspend the cell pellet in 1 ml of fresh Thawing Medium.
8. Transfer contents to the T75 tissue culture flask containing pre-equilibrated Thawing Medium and place flask in the humidified 37°C/5% CO_2 incubator.
9. At first passage, switch to Growth Medium.

5.2 Propagation Method

1. Passage or feed cells at least twice a week. Maintain cells between 5% and 95% confluence. Do not allow cells to reach confluence.
2. To passage cells, aspirate medium, rinse once in PBS, add 0.05% Trypsin/EDTA (3 ml for a T75 flask, 5 ml for a T175 flask, and 7 ml for T225 flask) and swirl to coat the cells evenly. Cells usually detach after ~2–5 minutes exposure to 0.05% Trypsin/EDTA. Add an equal volume of Growth Medium to inactivate the 0.05% Trypsin/EDTA.
3. Verify under a microscope that cells have detached and clumps have completely dispersed.
4. Centrifuge cells at 200 × g for 5 minutes and resuspend in Growth Medium.

5.3 Freezing Method

1. Harvest the cells as described in **Subsection 5.2** (above), Step 2. After detachment, count the cells, centrifuge cells at 200 × *g* for 5 minutes, and resuspend in 4°C Freeze Medium to a density of 2E6 cells/ml.
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 storage.

6. Media Requirements

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

Note: Make **NO MEDIA SUBSTITUTIONS**, as these cell lines have been specifically validated for optimal assay performance with these media. For dividing cells, we recommend that you create and store an aliquot for back up.

Component	Assay Medium	Growth Medium	Thawing Medium	Freeze Medium
DMEM	90%	90%	90%	—
Dialyzed FBS (Do not substitute!)	10%	10%	10%	—
NEAA	0.1 mM	0.1 mM	0.1 mM	—
HEPES (pH 7.3)	25 mM	25 mM	25 mM	—
Penicillin (antibiotic)	100 U/ml	100 U/ml	100 U/ml	—
Streptomycin (antibiotic)	100 µg/ml	100 µg/ml	100 µg/ml	—
Recovery™ Cell Culture Freezing Medium	—	—	—	100%
Zeocin™	—	100 µg/mL	—	—
Geneticin®	—	500 µg/mL	—	—

7. Transient Transfection Procedure and Agonist Test Assay

The following procedure describes how to transfect your Gi/o GPCR of interest into this Gqo5-containing cell line and then perform an assay using a known agonist to test for a positive beta-lactamase response from the NFAT response element. The parental cell line should be tested separately for any endogenous response to the agonist, as described in **Section 8**.

Note: **Gqo5 may not couple to all Gi/o GPCRs.** To determine if the chimeric Gqo5 protein is coupling your Gi/o GPCR of interest to the NFAT-*bla* reporter construct, it is necessary for the Response Ratio of the cell line containing the GPCR to be statistically different (*e.g.,* using a Student's T-test) than that of the parental cell line. The Response Ratio is defined as the Blue/Green ratio of maximally stimulated cells divided by the Blue/Green ratio of unstimulated cells (see **Section 10, Data Analysis**). A Response Ratio of ≥1.5 for the transiently transfected cell line generally indicates that it is a suitable candidate for the creation of a stable cell line.

The transfection procedure uses Lipofectamine™ 2000, available separately from Invitrogen (see **Section 4**). The substrate loading procedure uses LiveBLAzer™-FRET B/G Substrate Mixture (CCF4-AM), also available separately (see **Section 4**). If you are using alternative substrates (*e.g.,* ToxBLAzer™ DualScreen or LyticBLAzer™ Loading kits), follow the loading protocol provided with the product.

7.1 Quick Assay Reference Guide

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

	Unstimulated Wells	Stimulated Wells	Cell-free Wells
Step 1 Plate cells	90 µl cells in Growth Medium (25,000 cells/well)	90 µl cells in Growth Medium (25,000 cells/well)	90 µl Growth Medium (no cells)
Step 2 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 16–24 hours		
Step 3 Transfect cells	Aspirate Growth Medium and replace with 75 µl Transfection Mix.		
Step 4 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 16–20 hours		
Step 5 Remove Transfection Mix	Aspirate Transfection Mix and replace with 90 µl Growth Media.		
Step 6 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 16–24 hours		
Step 7 Add Agonist or Test Compounds	10 µl Assay Medium	10 µl 10X Agonist in Assay Medium	10 µl Assay Medium
Step 8 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 4-6 hours		
Step 9 Prepare 6X Substrate Mix	6 µl of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) Substrate + 60 µl of Solution B, mix. Add 904 µl of Solution C, mix. Add 30 µl of Solution D, mix.		
Step 10 Add Substrate Mixture	20 µl per well		
Step 11 Incubate Substrate Mix. + cells	2 hours at room temperature in the dark		
Step 12 Detect activity	See **Section 9**		
Step 13 Analyze data	See **Section 10**		

7.2 Detailed Assay Protocol

We suggest using a minimum of 15 wells for the Transfected/Stimulated condition, the Transfected/Unstimulated condition, and the Cell-free Control.

7.2.1 Precautions

- Work on a dust-free, clean surface. Always handle the 96-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

Day 1 (Plate the cells the day before the transfection):

1. Harvest cells and resuspend in Growth Medium (+) at a density of 2.7×10^5 cells/ml.
2. Add 90 µl per well of the Growth Medium (+) to the Cell-free Control wells. Add 90 µl per well of the cell suspension to the Stimulated wells and Unstimulated Control wells.

Note: Plate cells the day before the transfection and place them in a humidified 37°C/5% CO_2 incubator overnight.

7.2.3 Transient Transfection Procedure

Days 2 and 3:

Use the following procedure to transfect your Gi/o GPCR of interest into mammalian cells in a 96-well format. This procedure will make enough Transfection Mix for 33 wells.

Note: The plasmid containing your Gi/o GPCR of interest should *not* contain a Zeocin™ or Geneticin® resistance gene. We recommend using pcDNA™6.2-hygro-DEST Vector (see **Section 4** for ordering information).

1. **For each transfection sample**, prepare complexes as follows:
   a. Dilute 4 µg of the Gi/o coupled GPCR expression plasmid DNA in 250 µl of Opti-MEM® I Reduced Serum Medium (or other medium without serum). Mix gently.
   b. Mix Lipofectamine™ 2000 gently before use, then dilute 10 µl in 250 µl of Opti-MEM® I Medium. Incubate for 5 minutes at room temperature.

   Note: Proceed to Step C within 25 minutes.

   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine™ 2000 (total volume = 500 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

   Note: Complexes are stable for 6 hours at room temperature.
2. Add the 500 µl of DNA-Lipofectamine™ 2000 complexes to 2 ml of Growth Medium (–). This is the transfection mix.
3. Remove Growth Medium from the wells of the plate and replace with 75 µl of Transfection Mix per well. Incubate the assay plate in a humidified 37°C/5% CO_2 incubator for 16–20 hours.
4. Replace the Transfection Mix after 16–20 hours with 90 ml of Growth Medium (+) per well. Incubate the assay plate in a humidified 37°C/5% CO_2 incubator for another 16–24 hours for high-level expression of the GPCR.

7.2.4 Agonist Test Assay Plate Setup

Day 4:

Following transfection, prepare an assay using a known agonist to test for a positive beta-lactamase response from the NFAT response element.

1. Prepare a 10X stock of a known agonist in Assay Medium.
2. Add 10 µl of the 10X stock of agonist to the Stimulated wells.
3. Add 10 µl of the Assay Medium alone to the Unstimulated Control wells and the Cell-free Control wells.
4. Incubate the agonist assay plate in a humidified 37°C/5% CO_2 incubator for 4–6 hours.

7.2.5 Substrate Loading

Prepare the LiveBLAzer™-FRET B/G Substrate Mixture (CCF4-AM) and load the 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 by adding 912 µl of DMSO per mg of dry substrate. 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.
2. Prepare 6X Loading Solution:
   a. Add 6 µl of Solution A to 60 µl of Solution B and vortex.
   b. Add 904 µl of Solution C to the above solution and vortex.
   c. Add 30 µl of Solution D to the above solution and vortex.
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 20 µl (transient transfection assay plate) or 8 µl (parental cell negative control assay plate) 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. Proceed to **Section 9**, **Detection**.

7.3 Creating a Stable Cell Line

An antibiotic-selected stable pool of cells can be created by selection with hygromycin. We recommend using a concentration of 100 µg/ml of hygromycin for selection. Upon creation of a stable pool of cells containing the GPCR of interest, we recommend that you utilize flow cytometry to rapidly and quantitatively isolate a single clone with the desired performance characteristics (*e.g.,* maximum response ratio or moderate constitutive activity) for your experimental requirements.

8. Parental Cell Negative Control Assay Procedure

Use the following procedure to determine the activity of agonist compounds on the parental cell line. The following procedure uses LiveBLAzer™-FRET B/G Substrate Mixture (CCF4-AM), available separately from Invitrogen (see **Section 4**). If you are using alternative substrates (*e.g.*, ToxBLAzer™ DualScreen or LyticBLAzer™ Loading kits), follow the loading protocol provided with the product.

8.1 Quick Assay Reference Guide

For a more detailed assay protocol, see **Section 8.2**.

	Unstimulated Wells	Stimulated Wells	Cell-free Wells	Test Compound Wells
Step 1 Plate cells, incubate	32 µl cells in Assay Medium (5,000 cells/well)	32 µl cells in Assay Medium (5,000 cells/well)	32 µl Assay Medium (no cells)	32 µl cells in Assay Medium 5,000 cells/well)
	Incubate cells for 16–24 hrs. at 37°C/ 5%CO_2			
Step 2 Add Agonist or Test Compounds	8 µl Assay Medium with 0.5% DMSO	8 µl 5X agonist 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 3 Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 5 hours			
Step 4 Prepare 6X Substrate Mix	6 µl of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) Substrate + 60 µl of solution B, mix. Add 904 µl of Solution C, mix. Add 30 µl of Solution D, mix.			
Step 5 Add Substrate Mixture	8 µl per well			
Step 6 Incubate Substrate Mix. + cells	2 hours at room temperature in the dark			
Step 7 Detect activity	See **Section 9**			
Step 8 Analyze data	See **Section 10**			

8.2 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.

8.2.1 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.

8.2.2 Plating Cells

1. Harvest cells and resuspend in Assay Medium to a density of 156,000 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. Incubate cells at 37°C/5% CO_2 for 16–24 hours.

8.2.3 Assay Plate Setup

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.
3. Prepare a 5X stock of agonist in Assay Medium with 0.5% DMSO. We recommend running a dose response curve to determine the optimal concentration of the agonist 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 agonist 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 5 hours.

8.2.4 Substrate Loading

The following procedure uses LiveBLAzer™-FRET B/G Substrate Mixture (CCF4-AM), available separately from Invitrogen (see **Section 4**). If you are using alternative substrates (*e.g.,* ToxBLAzer™ DualScreen or LyticBLAzer™ Loading kits), follow the loading protocol provided with the product.

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 by adding 912 µl of DMSO per mg of dry substrate. Store the aliquots of the stock solution at –20°C until use. The molecular weight of the LiveBLAzer™-FRET B/G Substrate (CCF4-AM) is 1096 g/mol.
2. Prepare 6X Loading Solution:
   a. Add 6 µl of Solution A to 60 µl of Solution B and vortex.
   b. Add 904 µl of Solution C to the above solution and vortex.
   c. Add 30 µl of Solution D to the above solution and vortex.
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 20 µl (transient transfection assay plate) or 8 µl (parental cell negative control assay plate) 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. Proceed to **Section 9, Detection**.

9. Detection

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

9.2.1 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

9.2.2 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

10. Data Analysis

10.1 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 values by the background-subtracted Green emission values.

10.2 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).

11. 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.
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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.

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