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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

<b>Cell Line Name:</b>	TrkA-NFAT- <i>bla</i> CHO-K1
<b>Description:</b>	CellSensor® TrkA-NFAT- <i>bla</i> CHO-K1 cells contain a beta-lactamase reporter gene under control of the NFAT Response Element that has been stably integrated into CHO-K1 cells along with TrkA. This cell line is a clonal population isolated by flow cytometry that has been tested for robust assay performance by assessing a variety of assay parameters. TrkA-NFAT- <i>bla</i> CHO-K1 cells express beta-lactamase upon stimulation with nerve growth factor 2.5s (NGF 2.5s).
<b>Product Number:</b>	K1516
<b>Shipping Condition:</b>	Dry Ice
<b>Storage Condition:</b>	Liquid nitrogen. <b>Immediately upon receipt, cells must be stored in liquid nitrogen or thawed for immediate use. Cells stored at -80°C can quickly lose viability.</b>
<b>Quantity:</b>	~2,000,000 (2 × 10 <sup>6</sup> cells/mL)
<b>Application:</b>	Detection of agonists/antagonists of the NGF/TrkA signaling pathway.
<b>Growth Properties:</b>	Adherent
<b>Cell Phenotype:</b>	Epithelial
<b>Selection Marker:</b>	Blasticidin (5 µg/mL) and Zeocin™ (200 µg/mL)
<b>Vector Used:</b>	pcDNA-NFAT- <i>zeo</i> and pcDNA6.2/TrkA
<b>Mycoplasma Testing:</b>	Negative
<b>BioSafety Level:</b>	1

**For Technical Support for this or other Invitrogen Discovery Sciences Products, dial 760 603 7200, option 3, extension 40266**

### 3. Materials Required, but Not Supplied

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
DMEM + GlutaMax™-1	Invitrogen	10569-010
Opti-MEM® I Reduced Serum Medium	Invitrogen	11058-021
DMSO	Fluka	41647
Dialyzed Fetal Bovine Serum (dFBS) ( <b>DO NOT SUBSTITUTE!</b> )**	Invitrogen	26400-044
Nonessential amino acids (NEAA)	Invitrogen	11140-050
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140-122
Sodium Pyruvate	Invitrogen	11360-070
HEPES (1M, pH 7.3)	Invitrogen	15630-080
Phosphate-buffered saline without calcium and magnesium [PBS(-)]	Invitrogen	14190-144
0.05% Trypsin/EDTA	Invitrogen	25300-054
Nerve Growth Factor (NGF) 2.5s Murine, Natural	Invitrogen	13257-019
K252A Inhibitor	Invitrogen	PHZ1131
Blasticidin (antibiotic)	Invitrogen	R210-01
Zeocin™ (antibiotic)	Invitrogen	R250-01
Solution D	Invitrogen	K1156
BSA, 10% Stock Solution	Invitrogen	P2489

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 6.3)	Chroma Technology Corp.
<b>Optional:</b> Epifluorescence- or fluorescence-equipped microscope with appropriate filters	Various
<b>Optional:</b> Microplate centrifuge	Various

**Note:** If you do not have access to a fluorescence plate reader with bottom-read capabilities, contact our Technical Support for options of other beta-lactamase substrates that can be read with top-reading instruments. 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.

\* Some part numbers differ outside of the continental US. Please check with your local Invitrogen Technical Support.

\*\* 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.

## 4. Media Requirements

Component	Thaw Medium	Growth Medium	Assay Medium*	Freezing Medium
DMEM + GlutaMax™-1	500 mL bottle	500 mL bottle	—	—
Opti-MEM® I Medium	—	—	500 mL bottle	—
Dialyzed Fetal Bovine Serum (dFBS)	50 mL	50 mL	2.5 mL	—
NEAA (10 mM)	5 mL	5 mL	5 mL	—
Sodium Pyruvate (100 mM)	—	—	5 mL	—
HEPES (pH 7.3, 1 M)	12.5 mL	12.5 mL	—	—
Penicillin (10,000 U/mL) / Streptomycin (10,000 µg/mL)	5 mL	5 mL	5 mL	—
Blasticidin	—	5 µg/mL	—	—
Zeocin	—	200 µg/mL	—	—
Recovery™ Cell Culture Freezing Medium	—	—	—	100%

**\*Note:** An additional assay medium formulation containing lower serum concentration has also been tested and shown to perform comparably well with this cell line: DMEM + GlutaMax™-1 (500 mL bottle) supplemented with 0.5 mL dFBS, 5 mL NEAA (10 mM stock), 12.5 mL HEPES (pH7.3, 1 M stock), and 5 mL of Penicillin (10,000 U/mL)/Streptomycin (10,000 µg/mL).

**Note:** We prepare our media by adding the listed components directly to the medium bottle. Blasticidin and Zeocin can be added directly to the cell culture flask to reach 5 µg/mL and 200 µg/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.

## 5. Detailed Cell Handling Procedures

### 5.1 Thawing Method

- Place 14 mL of Thaw Medium (without Blasticidin and Zeocin) into a T75 flask.
- Place the flask in a humidified 37°C/5% CO<sub>2</sub> incubator for 15 minutes to allow medium to equilibrate to the proper pH and temperature.
- 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 vial in water.
- Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
- Transfer the vial contents to a sterile 15-mL conical tube.
- Add 10 mL of Thaw Medium (without Blasticidin and Zeocin) dropwise into the cell suspension.
- Centrifuge cells at 200 × *g* for 5 minutes.
- Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh Thaw Medium (without Blasticidin and Zeocin).

**Note:** This step is important to fully remove the DMSO present from the Recovery™ Cell Culture Freezing Medium.

- Transfer contents to the T75 tissue culture flask containing pre-equilibrated Thaw Medium (without Blasticidin and Zeocin) and place flask in a humidified 37°C/5% CO<sub>2</sub> incubator.
- Switch to passaging cells in Growth Medium with Blasticidin and Zeocin, once cells appear to be growing at consistent rates for the given cellular background. See **Section 5.4** for special considerations.

## 5.2 Propagation Method

1. Cells should be passaged or fed at least two times a week. Cells should be maintained between 10% and 90% confluency. Do not allow cells to reach confluence. Cells which have grown to confluence may not show expected agonist response in the assay.
2. To passage cells, aspirate medium, rinse once with PBS, add Trypsin/EDTA (3 ml for a T75 flask, 5 ml for a T175 flask and 8 ml for T225 flask) and swirl to coat the cells evenly. Cells usually detach after ~2 minutes exposure to Trypsin/EDTA. Add Growth Medium (7 ml for a T75 flask, 10 ml for T175 and T225 flasks) to inactivate Trypsin and mix. Verify under a microscope that cells have detached and clumps have completely dispersed.
3. Transfer required amount to a new flask containing prewarmed Growth Medium.

## 5.3 Freezing Method

1. Harvest and count the cells, then spin cells down and resuspend in 4°C Recovery™ Cell Culture Freezing Medium at a density of  $2 \times 10^6$  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.

## 5.4 Special Considerations

- This cell line is a clonal population isolated by Fluorescence Activated Cell Sorting (FACs) based on the functional response to NGF 2.5s. Assay performance can be expected to depend upon use of the specified media as responsive cells have been chosen based on these formulations.
- For additional information about the NFAT-*bla* CHO-K1 cellular background please contact Technical Support at 1-760-603-7200, select option 3 and enter extension 40266.
- Cryopreserved TrkA-NFAT-*bla* CHO-K1 cells have been tested to be responsive to NGF 2.5s. For more information on cryopreserved cell provision services and using cryopreserved cells directly for the assay, please contact Technical Support.
- This cell line is Blastocidin and Zeocin resistant.

## 6. Assay Procedure

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The following instructions outline the recommended procedure for monitoring NGF-induced response 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 7**.
- 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](http://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.

## 6.1 Quick Reference Guide

For more detailed protocol information, see [Section 6.2](#).

### Agonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Cell-free Wells	Test Compound Wells
<b>Step 1</b> Plate cells	32 µl cells in Assay Medium (10,000 cells/well)	32 µl cells in Assay Medium (10,000 cells/well)	32 µl Assay Medium (no cells)	32 µl cells in Assay Medium (10,000 cells/well)
<b>Step 2</b> Add Agonist or Test Compounds	8 µl Assay Medium with 0.5% DMSO	8 µl 5X NGF in Assay Medium with 0.5% DMSO	8 µl Assay Medium with 0.5% DMSO	8 µl 5X Test Compounds in 0.5% DMSO
<b>Step 3</b> Incubate cells	Incubate in a humidified 37°C/5% CO <sub>2</sub> incubator for 5 hours			
<b>Step 4</b> Prepare 6X Substrate Mix	6 µl of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) Substrate + 60 µl of solution B, mix. Add 874 µl of Solution C and 60 µl of Solution D, mix			
<b>Step 5</b> Add Substrate Mixture	8 µl per well			
<b>Step 6</b> Incubate	2 hours at room temperature in the dark			
<b>Step 7</b> Detect activity	See <a href="#">Section 6.3</a>			
<b>Step 8</b> Analyze data	See <a href="#">Section 7</a>			

### Antagonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Control Compound Wells	Cell-free Wells	Test Compound Wells
<b>Step 1</b> Plate cells	32 µl cells in Assay Medium (10,000 cells/well)	32 µl cells in Assay Medium (10,000 cells/well)	32 µl cells in Assay Medium (10,000 cells/well)	32 µl Assay Medium (no cells)	32 µl cells in Assay Medium (10,000 cells/well)
<b>Step 2</b> Add Antagonist or Test Compounds	4 µl Assay Medium with 1% DMSO	4 µl Assay Medium with 1% DMSO	4 µl 10X K252A Inhibitor in Assay Medium with 1% DMSO	4 µl Assay Medium with 1% DMSO	4 µl 10X Test Compounds in Assay Medium with 1% DMSO
<b>Optional Step:</b>	Incubate plate with Antagonist for 30 minutes before proceeding				
<b>Step 3</b> Add Agonist	4 µl Assay Medium	4 µl 10X NGF in Assay Medium	4 µl 10X NGF in Assay Medium	4 µl 10X NGF in Assay Medium	4 µl 10X NGF in Assay Medium
<b>Step 4</b> Incubate cells	Incubate in a humidified 37°C/5% CO <sub>2</sub> incubator for 5 hours				
<b>Step 5</b> Prepare 6X Substrate Mix	Add 6 µl of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) substrate + 60 µl of solution B, mix. Add 874 µl of Solution C and 60 µl of Solution D, mix				
<b>Step 6</b> Add Substrate Mixture	8 µl per well				
<b>Step 7</b> Incubate	2 hours at room temperature in the dark				
<b>Step 8</b> Detect activity	See <a href="#">Section 6.3</a>				
<b>Step 9</b> Analyze data	See <a href="#">Section 7</a>				

## 6.2 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.

### 6.2.1 Precautions and Special Considerations for this Assay

- 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 (30 seconds at  $14 \times g$ ) after additions to ensure all assay components are on the bottom of the wells.
- Cells should be grown to a confluency between 50% to 75% before harvesting and plating for the assay.

### 6.2.2 Plate Cells

1. Harvest cells from culture in Growth Medium and resuspend in Assay Medium to a density of 312,500 cells/mL.
2. Add 32  $\mu$ L per well of Assay Medium to the Cell-free control wells. Add 32  $\mu$ L per well of the cell suspension to the Test Compound wells, the Unstimulated Control wells, and Stimulated Control wells.
3. Incubate the plates in a 37°C/5% CO<sub>2</sub> incubator for 16 hours.
4. Proceed to **Section 6.2.3** for an Agonist assay or **Section 6.2.4** for an Antagonist assay.

### 6.2.3 Agonist Assay Plate Setup

**Note:** This subsection provides directions for performing an Agonist assay. See **Section 6.2.4** for directions for performing an Antagonist assay.

**Note:** The positive agonist controls are run at the concentration of NGF 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 NGF solution. See **Section 7.3** 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%).

**Note:** NGF 2.5s is supplied by the manufacturer at a concentration of 100  $\mu$ g/mL (3.85  $\mu$ M) in 100  $\mu$ L of 10 mM sodium acetate (pH 5). Aliquots can be stored at -20°C for long-term storage (several months). NGF is freeze/thaw sensitive, once thawed, stored at 4°C for up to 1 week.

3. Prepare a 5X stock of NGF in Assay Medium containing 0.5% DMSO. For maximum stimulation, use ~4.5 nM final concentration (5X = 22.5 nM). We recommend running a dose response curve to determine the EC<sub>100</sub> for your NGF solution. See **Section 7.3** for a representative curve.
4. Add 8  $\mu$ L of the stock solution of 0.5% DMSO in Assay Medium to the Unstimulated Control and Cell-free Control wells.
5. Add 8  $\mu$ L of the 5X stock solution of NGF to the Stimulated Control wells.
6. Add 8  $\mu$ L 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<sub>2</sub> incubator for 5 hours. Then proceed to **Section 6.2.5** for Substrate Loading and Incubation.

### 6.2.4 Antagonist Assay Plate Setup

**Note:** This subsection provides directions for performing an Antagonist assay. See **Section 6.2.3** 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%).

**Note:** NGF 2.5s is supplied by the manufacturer at a concentration of 100  $\mu$ g/mL (3.85  $\mu$ M) in 100  $\mu$ L of 10 mM sodium acetate (pH 5). Aliquots can be stored at -20°C for long-term storage (several months). NGF is freeze/thaw sensitive, once thawed, stored at 4°C for up to 1 week.

3. Prepare a 10X stock of NGF in Assay Medium at an EC<sub>80</sub> concentration. We recommend running a dose response curve to determine the EC<sub>80</sub> for your NGF solution. See **Section 7.3** for a representative curve. In this example the final EC<sub>80</sub> concentration, was 0.47 nM, (10X = 4.7 nM).
4. Prepare a 10X stock of K252A Inhibitor (control antagonist compound) in Assay Medium with 1.0% DMSO. For maximum inhibition, use 1.0  $\mu$ M final concentration (10X = 10  $\mu$ M). We recommend running a dose response curve to determine the optimal inhibition concentration for the Antagonist solution.
5. Add 4  $\mu$ L of the 10X stock of Test Compounds to the Test Compound wells.
6. Add 4  $\mu$ L of the stock solution of 1.0% DMSO to the Stimulated Control wells, the Unstimulated Control wells, and the Cell-free Control wells.

7. Add 4 µl of the 10X stock of K252A Inhibitor to the Antagonist Control wells.
8. If desired, incubate the Test Compounds with the cells in a humidified 37°C/5% CO<sub>2</sub> incubator before proceeding. Typically, a 30-minute incubation is sufficient.
9. Add 4 µl of the 10X EC<sub>80</sub> stock solution of NGF prepared in step 4 to the Test Compound wells, the Stimulated Control wells, and the Antagonist Control wells.
10. Add 4 µl of Assay Medium to the Unstimulated Control and Cell-free Control wells.
11. Incubate the Antagonist assay plate in a humidified 37°C/5% CO<sub>2</sub> incubator for 5 hours. Then proceed to **Section 6.2.5** for Substrate Loading and Incubation.

### 6.2.5 Substrate Loading and Incubation

This protocol is designed for loading cells with LiveBLAzer™-FRET B/G Substrate (CCF4-AM). If using ToxBLAzer™ substrate, prepare and utilize as you would LiveBLAzer.

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

1. Prepare Solution A: 1 mM LiveBLAzer™-FRET B/G Substrate (CCF4-AM, MW = 1096) stock solution:
  - 1.1 Add anhydrous DMSO directly to the vial of lyophilized CCF4-AM, using 182 µL of DMSO for every 200 µg of CCF4-AM.
  - 1.2 Mix well.
  - 1.3 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 874 µL Solution C and 60 µL Solution D 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<sub>2</sub> incubator and allow plate to equilibrate to room temperature.
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 hours.

## 6.3 Detection

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

### 6.3.1 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](http://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.
- If using ToxBLAzer™, refer to that protocol for specific instrumentation and filter recommendations.

### 6.3.2 Reading an Assay Plate

1. 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: 405 nm excitation & 460 nm emission; measurement 2: 405 nm excitation & 530 nm emission). Some instrument settings may also require optimization (e.g. gain, plate height, flash number, integration time, etc.).

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

## 7. Data Analysis

### 7.1 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 response ratio to know your assay window. The response ratio is calculated as the Blue/Green Emission Ratio of the NGF –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  $Z' \geq 0.5$ .

### 7.2 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 is 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](http://www.chroma.com)).

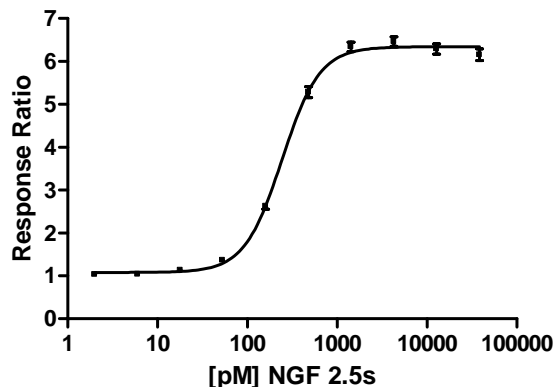
#### Chroma Set # 41031

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).



### 7.3 Representative Data



<b>EC<sub>50</sub></b>	243.3 pM (6.33 ng/ml)
<b>EC<sub>80</sub></b>	474.3 pM (12.33 ng/ml)
<b>EC<sub>100</sub></b>	~ 4280 pM (111 ng/ml)
<b>Z' at EC<sub>100</sub></b>	0.75

**Figure 1. Dose response of TrkA-NFAT-*bla* CHO-K1 cells to NGF 2.5s.** TrkA-NFAT-*bla* CHO-K1 cells were plated in 384-well format and then stimulated with NGF 2.5s (Invitrogen # 13257-019) 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 (CCF4-AM) plus Solution D for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader. The response ratio was calculated as the Blue/Green Emission Ratio of the NGF 2.5s-stimulated wells divided by the Blue/Green Emission Ratio of the unstimulated wells and plotted against the NGF 2.5s concentration (n = 16 for each data point).

## 8. 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.

## 9. Purchaser Notification

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