Tango™ CXCR2-*bla* U2OS DA and Dividing Cell-based Assay

invitrogen™

Cat. nos. K1769 and K1521 | **Shipping: Dry Ice** | **Storage: Liquid Nitrogen**

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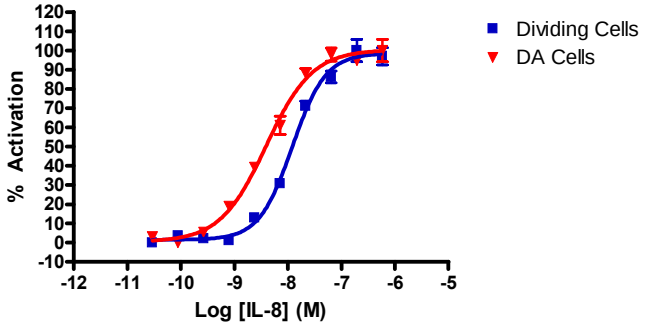
1. Description

Tango™ CXCR2-*bla* U2OS DA (division-arrested) cells and dividing cells contain the human Chemokine (C-X-C motif) receptor 2 linked to a TEV protease site and a Gal4-VP16 transcription factor stably integrated into the Tango™ GPCR-*bla* U2OS parental cell line. This parental cell line stably expresses a beta-arrestin/TEV protease fusion protein and the beta-lactamase reporter gene under the control of a UAS response element. The Tango™ CXCR2-*bla* U2OS cells have been functionally validated for a response to IL-8.



	Division-arrested cells	Dividing Cells
EC_{50}	4 nM	12 nM
Z′-factor at EC_{100}	0.74	0.59

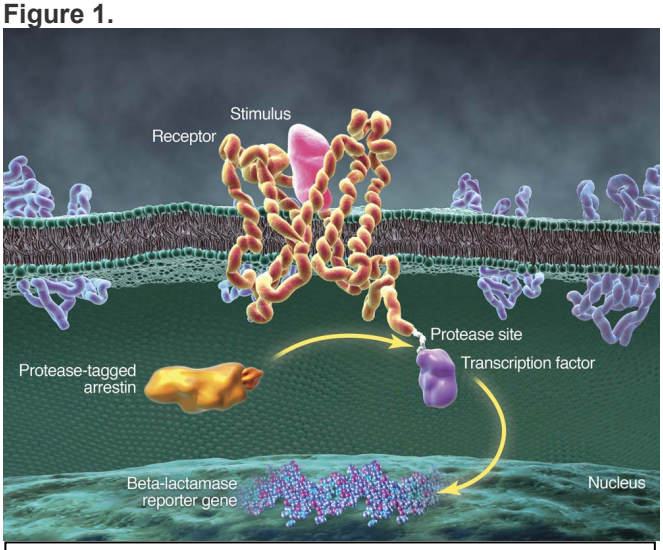
Dose response of Tango™ CXCR2-*bla* U2OS DA cells and dividing cells to IL-8.

2. Overview of Tango™ GPCR Cell Based Assays

The Tango™ GPCR Assay technology combines the benefits of the Tango™ assay platform with the highly accurate, sensitive, and easy to use GeneBLAzer® beta-lactamase (*bla*) reporter system. The Tango™ assay platform is based upon ligand binding to G-Protein Coupled Receptors (GPCRs) that triggers desensitization, a process mediated by the recruitment of intracellular arrestin proteins to the activated receptor. As a result, the ligand-induced activation of GPCRs may be assayed by monitoring the interaction of arrestin with the test GPCR. A major advantage of this approach is that it does not depend on knowledge of the G-protein signaling specificity of the target receptor.

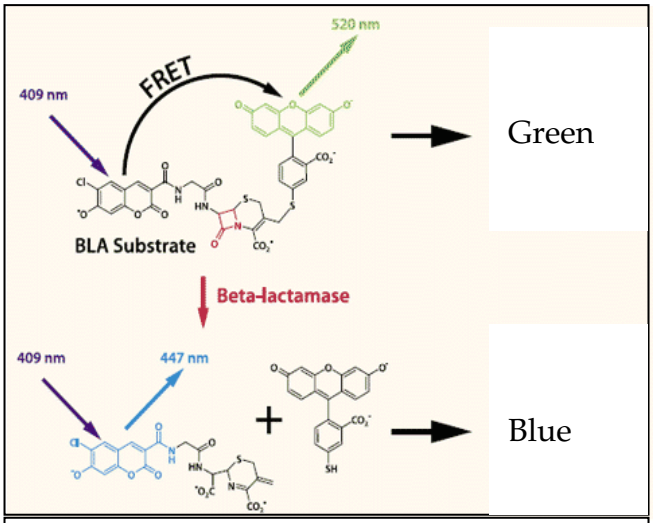
The design of the Tango GPCR assay is shown in Figure 1. The target GPCR is fused at its intracellular C-terminus to an exogenous transcription factor. Interposed between the receptor and the transcription factor is a specific cleavage sequence for a non-native protease. This chimeric receptor protein is expressed in a cell line containing the *bla* reporter gene responsive to the transcription factor. The cell line also expresses an arrestin-protease fusion protein that recognizes and cleaves the site between the receptor and transcription factor. The assay is performed by adding a ligand to the growing cells for a defined period and measuring the activity of the reporter gene. Activation of the reporter gene provides a quantifiable measurement of the degree of interaction between the target receptor and the protease-tagged arrestin partner. Additionally, it is unaffected by other signaling pathways in the cell, thus providing a highly selective readout of target receptor activation.

Figure 1.



Upon ligand binding and receptor activation, a protease-tagged beta-arrestin is recruited to the GPCR that has been modified at the C-terminus to include a transcription factor linked by a protease cleavage site. The protease in turn cleaves the transcription factor from the GPCR, the transcription factor immediately translocates to the nucleus, and beta-lactamase activity is activated.

Figure 2.



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.

The Tango™ 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 2). 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.

The resulting coumarin:fluorescein ratio provides a normalized reporter response that can minimize experimental noise that masks subtle underlying biological responses of interest. The Tango™ beta-lactamase reporter technology has been proven in high-throughput screening (HTS) campaigns for a range of target classes, including GPCRs (2, 3), nuclear receptors (4-6) and kinase signaling pathways (7). The utility of division-arrested cells in HTS has also been demonstrated (8-11).

3. Overview of Dividing and Division-Arrested Cells

Many of Invitrogen's cell lines are available in two forms: dividing or division arrested. Invitrogen's division-arrest technology allows the use of frozen cells, made from the exact same cell line sold in its dividing form, as ordinary, cost-effective assay reagents for screening. Division-arrested (DA) cells exhibit response profiles similar to those of dividing cells, thus ensuring that you obtain the correct pharmacological profile.

DA cells may be plated and assayed within 24 hours of thawing. Cell numbers for DA cells increase only marginally after plating, thereby removing the variability caused by cell division during the course of an assay and providing more consistent results.

4. Materials Supplied

Product: (One of these)	Name	Size	Catalog #
	Tango™ CXCR2-*bla* U2OS DA Assay Kit Each system contains sufficient division-arrested cells and substrate to assay one 384-well plate. Includes: • Tango™ CXCR2-*bla* U2OS DA cells (K1769A) • LiveBLAzer™-FRET B/G Loading Kit, 70 µg • Solution D, 1 ml • Protocol • Certificate of Analysis	1 plate	K1769
	Tango™ CXCR2-*bla* U2OS cells Includes: • Tango™ CXCR2-*bla* U2OS cells (K1521A) • Protocol • Certificate of Analysis	1 tube	K1521
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 Non-Division-arrested Cells:	Adherent		
Cell Phenotype:	Epithelial		
Selection Marker(s) for Non-Division arrested cells:	Zeocin™ 200 µg/ml, Geneticin® 100 µg/ml, and Hygromycin 50 µg/ml		
***Mycoplasma* Testing:**	Negative		
BioSafety Level:	1		

5. Materials Required

Use the table below to determine the additional media and reagents required for use with each kit:

Media/Reagents	Recommended Source	Part #	Required Separately?	
			Tango™ CXCR2-*bla* U2OS DA Assay Kit (K1769)	Tango™ CXCR2-*bla* U2OS cells (K1521)
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)	No (included in kit)	Yes
Solution D	Invitrogen	K1156 (1 ml) K1157 (25 ml)	No (included in kit)	Yes
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648-010	No	Yes
FreeStyle™ Expression Medium	Invitrogen	12338-018	Yes	Yes
McCoy's 5A Medium (modified) (1X)	Invitrogen	16600-082	No	Yes
DMSO	Fluka	41647	Yes	Yes
Fetal bovine serum (FBS), dialyzed, (**DO NOT SUBSTITUTE!**)	Invitrogen	26400-036	NO	Yes
Non-essential amino acids (NEAA)	Invitrogen	11140-050	No	Yes
Penicillin/Streptomycin (antibiotics)	Invitrogen	15140-122	No	Yes
Sodium Pyruvate	Invitrogen	11360-070	No	Yes
Phosphate-buffered saline without calcium and magnesium [PBS(-)]	Invitrogen	14190-136	No	Yes
HEPES (1 M, pH 7.3)	Invitrogen	15630-080	No	Yes
0.05% Trypsin/EDTA	Invitrogen	25300-054	No	Yes
IL-8	Biosource (IVGN)	PHC0885	Yes	Yes
Zeocin™	Invitrogen	R250-01	No	Yes
Hygromycin	Invitrogen	10687-010	No	Yes
Geneticin®	Invitrogen	10131-027	No	Yes

The following table lists additional items required for use with all kits:

Consumables	Recommended Source	Part #
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 8.4.1**)	Chroma Technologies	

5.1 Optional Equipment and Materials

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

6. Detailed Cell Handling Procedures

Note: DA cells have different thawing procedures than dividing cells. Refer to the instructions below for your particular application.

Note: Refer to **Section 7, Media Requirements** for specific media recipes.

6.1 DA Cells Thawing Method

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 8.2.2, 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 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 1 ml fresh Assay Medium. (For the 1 x 384-well plate size of cells, dilute in 1 ml.)
6. Count the cells.
7. Adjust the cell density with Assay Medium to the appropriate cell density as specified in Section 7. Proceed to **Section 8, Assay Procedure**, for guidance on using cells in an assay.

6.2 Dividing Cells

6.2.1 Thawing Method

Note: Cells are shipped to you 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.

6.2.2 Propagation Method

1. Passage or feed cells at least twice a week. Maintain cells between 25% 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 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.

6.2.3 Freezing Method

1. Harvest the cells as described in **Subsection 6.2.2** (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.

7. 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 (DA and dividing cells)	Growth Medium (dividing cells only)	Thawing Medium (dividing cells only)	Freeze Medium (dividing cells only)
FreeStyle™ Expression Medium	100%	—	—	—
McCoy's 5A Medium	—	90%	90%	—
Dialyzed FBS (Do not substitute!)		10%	10%	—
NEAA	—	0.1 mM	0.1 mM	—
HEPES (pH 7.3)	—	25 mM	25 mM	—
Sodium Pyruvate	—	1 mM	1 mM	—
Penicillin (antibiotic)	—	100 U/ml	100 U/ml	—
Streptomycin (antibiotic)	—	100 µg/ml	100 µg/ml	—
Recovery™ Cell Culture Freezing Medium	—	—	—	100%
Zeocin™	—	200 µg/ml	—	—
Hygromycin	—	50 µg/ml	—	—
Geneticin®	—	100 µg/ml	—	—

8. Assay Procedure

The following instructions outline the recommended procedure for determining activity of compounds as modulators of CXCR2 using LiveBLAzer™-FRET B/G Substrate as the readout. If alternative substrates are used (*e.g.*, ToxBLAzer™ DualScreen or LyticBLAzer™ Loading kits), follow the loading protocol provided with the product.

Note: For convenience, dividing cells can be incubated for 16–20 hours or 48 hours prior to compound addition (Step 1 in the Quick Assay Reference Guide). Do not seed division arrested cells for a 16–20 hour incubation, as the response window may decrease.

8.1 Quick Assay Reference Guides

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

Agonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Cell-free Wells	Test Compound Wells
Step 1 Plate cells, incubate	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)
	Incubate cells for 48 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 8.4**			
Step 8 Analyze data	See **Section 9**			

Antagonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Antagonist Control Wells	Cell-free Wells	Test Compound Wells
Step 1 Plate cells, incubate	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)
	Incubate cells for 48 hrs. at 37°C/ 5%CO_2				
Step 2 Add Antagonist or Test Compounds, incubate	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
	Incubate plate with Antagonist for 30 minutes before proceeding				
Step 3 Add Agonist	4 µl Assay Medium with 0.5% DMSO	4 µl 10X agonist in Assay Medium with 0.5% DMSO	4 µl 10X agonist in Assay Medium with 0.5% DMSO	4 µl 10X agonist in Assay Medium with 0.5% DMSO	4 µl 10X agonist in Assay Medium with 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 904 µl of Solution C, mix. Add 30 µl of Solution D, mix.				
Step 6 Add Substrate Mixture	8 µl per well				
Step 7 Incubate Mixture	2 hours at room temperature in the dark				
Step 8 Detect activity	See **Section 8.4**				
Step 9 Analyze data	See **Section 9**				

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. Thaw DA cells/harvest dividing cells and resuspend in Assay Medium to a density of 312,500 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 48 hours. Proceed to **Section 8.2.3** for an Agonist assay or **Section 8.2.4** for an Antagonist assay. ***Note:*** *For convenience, dividing cells can be incubated for 16-20 hours or 48 hours prior to compound addition. Do not seed division arrested cells for a 16-20 hour incubation, as the response window may decrease.*

8.2.3 Agonist Assay Plate Setup

Note: This subsection provides directions for performing an Agonist assay. See **Section 8.2.4** 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.
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. Then proceed to **Section 8.3** for Substrate Loading and Incubation.

8.2.4 Antagonist Assay Plate Setup

Note: This subsection provides directions for performing an Antagonist assay. See **Section 8.2.3** 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.
3. Prepare a 10X stock of agonist in Assay Medium with 0.5% DMSO. We recommend running a dose response curve to determine the optimal agonist concentration. For antagonist assays, we recommend stimulating cells initially with an agonist concentration in the EC_{50}-EC_{80} range.
4. Prepare a 10X stock of antagonist in Assay Medium with 0.5% DMSO. 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 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 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 agonist 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 5 hours. Then proceed to **Section 8.3** for Substrate Loading and Incubation.

8.3 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 use alternative substrates, follow the loading protocol provided with the 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 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 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.

8.4 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.

8.4.1 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

8.4.2 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

9. Data Analysis

9.1 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.

9.2 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).

10. References

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11. Purchaser Notification

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information about purchasing a license to use this product or the technology embedded in it for any use other than for research use please contact Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008; Phone (760) 603-7200 or e-mail: outlicensing@lifetech.com.

Limited Use Label License No. 108: Lentiviral Technology

The Lentiviral Technology (based upon the lentikat™ system) is licensed from Cell Genesys, Inc., under U.S. Patent Nos. 5,834,256; 5,858,740; 5,994,136; 6,013,516; 6,051,427; 6,165,782 and 6,218,187 and corresponding patents and applications in other countries for internal research purposes only. Use of this technology for gene therapy applications or bioprocessing other than for non-human research use requires a license from Cell Genesys (Cell Genesys, Inc. 342 Lakeside Drive, Foster City, California 94404). The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity), including non-gene therapy research and target validation applications in laboratory animals.

Limited Use Label License No. 150: GeneBLAzer® Technology

This product and/or its use is the subject of one or more of U.S. Patent Nos. 5,741,657, 5,955,604, 6,291,162, and 6,472,205 and foreign equivalents licensed to Life Technologies Corporation. The right to use this product for internal research specifically excludes the right to use this product to identify, discover, and profile compounds that act as a flavor, fragrance or taste-enhancers and modify a target identified in taste, olfaction, or pheromone detection, which compound does not require FDA approval of an NDA for claims of safety and efficacy. The right to use methods claimed in the foregoing patents with this product for research purposes can only be acquired by the use of GeneBLAzer® substrates purchased from Life Technologies Corporation or its authorized distributors.

Limited Use Label License No. 315: Division Arrested Cell Products

The purchaser of this product may not remove or obtain components from cells sold under this label license or use these cells in a manner inconsistent with protocols provided by Life Technologies Corporation for use of division arrested cells. The purchaser of this product may not attempt to expand division arrested cells and may only use the purchased amount of this product.

Limited Use Label License No. 317: LentiVector® Technology

This product is licensed under U.S. Pat. Nos. 5,817,491; 5,591,624; 5,716,832; 6,312,682; 6,669,936; 6,235,522; 6,924,123 and foreign equivalents from Oxford BioMedica (UK) Ltd., Oxford, UK, and is provided for use in academic and commercial in vitro and in vivo research for elucidating gene function, and for validating potential gene products and pathways for drug discovery and development, but excludes any use of LentiVector® technology for: creating transgenic birds for the purpose of producing useful or valuable proteins in the eggs of such transgenic birds, the delivery of gene therapies, and for commercial production of therapeutic, diagnostic or other commercial products not intended for research use where such products do not consist of or incorporate a lentiviral vector. Information about licenses for commercial uses excluded under this license is available from Oxford BioMedica (UK), Ltd., Medawar Centre, Oxford Science Park, Oxford OX4 4GA UK enquiries@oxfordbiomedica.co.uk or BioMedica Inc 11622 El Camino Real #100, San Diego CA 92130- 2049 USA. LentiVector is a registered US and European Community trade mark of Oxford BioMedica plc.

Use of Genetically Modified Organisms (GMO)

Information for European Customers The Tango™ CXCR2-*bla* U2OS DA and Tango™ CXCR2-*bla* U2OS cell lines are genetically modified with the plasmids pTango™ β-Arr/TEV, pLenti-zeo/UAS-bla (note this construct was utilized as a plasmid not as a lentiviral stock) and pTangoCXCR2. 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.