Tango™ GPR119-*bla* U2OS DA and Dividing Cell-based Assay

Catalog Number K1777 and K1770

Doc. Part No. K1777.pps Pub. No. MAN0002970 Rev. 2.0

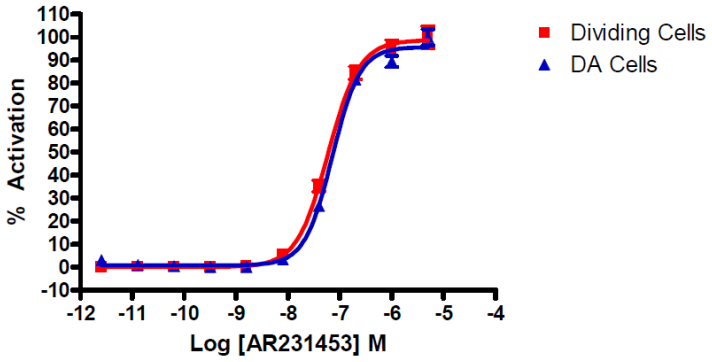
WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Tango™ GPR119-*bla* U2OS DA (division-arrested) cells and dividing cells contain the human G protein-coupled receptor 119 linked to a TEV protease site and a Ga14-VP16 transcription factor stably integrated into the Tango™ GPCR-*bla* U2OS 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™ GPR119-*bla* U2OS cells have been functionally validated for a response to AR231453.

Table 1 Dose response of Tango™ GPR119-*bla* U2OS division-arrested (DA) cells and dividing cells to AR231453

Parameter	DA cells	Dividing cells
EC_{50}	72.36 nM	59.83 nM
Z'-factor at EC_{100}	0.70	0.64



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 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.

The Tango™ assay technology uses a mammalian-optimized beta-lactamase (*bla*) reporter gene combined with a FRET-enabled substrate to provide reliable and sensitive detection in cells (Zlokarnik, 1998) (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™ assay technology has been proven in high-throughput screening (HTS) campaigns for a range of target classes, including G-protein coupled receptors (GPCRs) (Kunapuli, 2003; Xing, 2000), nuclear receptors (Qureshi, 2003; Peekhaus, 2003; Chin, 2003), and kinase signaling pathways (Whitney, 1998). The utility of division-arrested (DA) cells in HTS has also been demonstrated (Fursov, 2005; Kunapuli, 2005; Digan, 2005; Vasudevan, 2005).

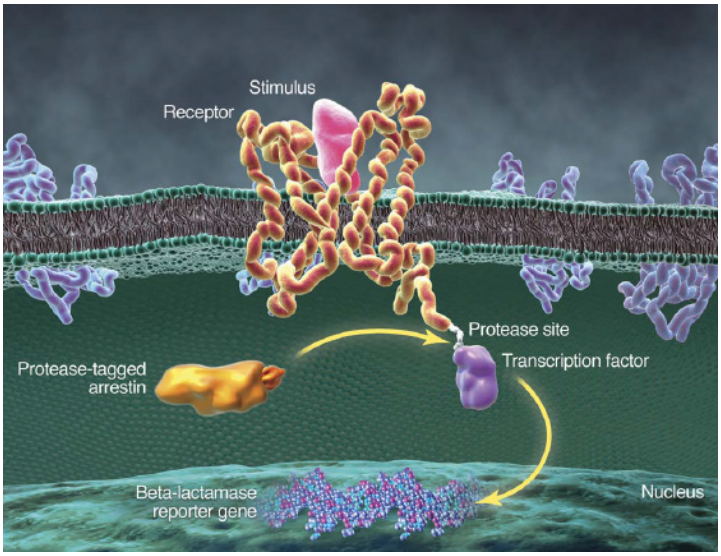


Figure 1 Tango™ GPCR assay design

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.

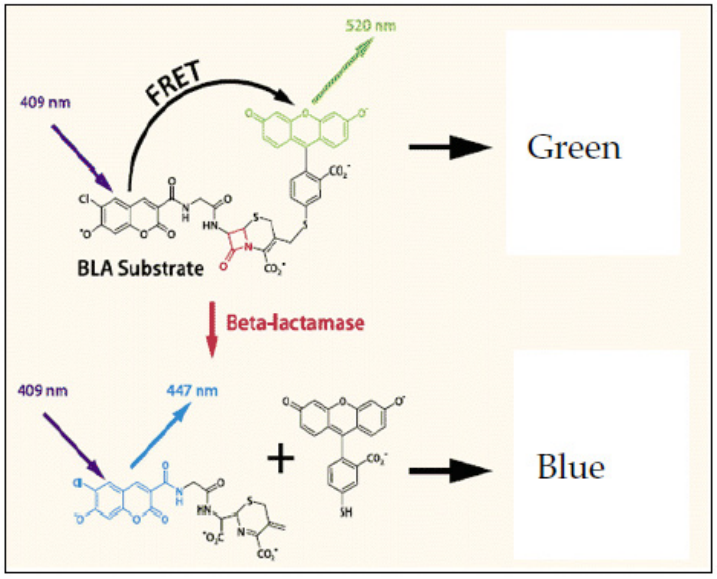


Figure 2 Fluorescent detection of beta-lactamase reporter gene response using a FRET-enabled substrate

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.

Overview of dividing and division-arrested cells

Many Invitrogen™ cell lines are available in two forms: dividing or division-arrested (DA). Invitrogen™ 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. DA cells exhibit response profiles similar to those of dividing cells, thus ensuring that you obtain the correct pharmacological profile.

DA cells can 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.

Contents and storage

Note: Cat. No. K1770 and K1777 are sold as separate products.

Table 2 Tango™ GPR119-*bla* U2OS DA Assay Kit (Cat. No. K1770)

Contents	Cat. No.	Amount[1]	Storage
Tango™ GPR119-*bla* U2OS DA cells	K1770A	1 tube	Liquid nitrogen, vapor phase
LiveBLAzer™-FRET B/G Loading Kit (70 µg) that includes the following reagents:	K1427[2]	1 kit	
LiveBLAzer™-FRET B/G Substrate (CCF4-AM) (Cat. No. K1089)[3]			–20°C
DMSO for Solution A			Room temperature
Solution B			Room temperature
Solution C			Room temperature
Solution D	K1156	1 mL	Room temperature
Protocol and Certificate of Analysis	—		

[1] Each system contains sufficient DA cells and substrate to assay one 384-well plate.
[2] Additional kit sizes are available.
[3] Cat. No. K1089 can be purchased separately from the kit.

Table 3 Tango™ GPR119-*bla* U2OS cells (Cat. No. K1777)

Contents	Cat. No.	Amount	Storage
Tango™ GPR119-*bla* U2OS cells	K1777A	1 tube	Liquid nitrogen, vapor phase
Protocol and Certificate of Analysis	—		

IMPORTANT! Cells are shipped overnight on dry ice. Immediately upon receipt, either thaw for immediate use or store in liquid nitrogen, vapor phase. Cells stored for more than 1 day at –80°C quickly lose viability.

Product details

Feature	Details
Shipping condition	Dry ice overnight
Growth properties of dividing cells	Adherent
Cell phenotype	Epithelial
Selection marker(s) for dividing cells	• Zeocin™ 200 µg/mL • Geneticin™ 100 µg/mL • Hygromycin-B 50 µg/mL
***Mycoplasma* testing**	Negative
Biosafety level	1

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 4 Required materials for dividing cells (Cat. No. K1777) and DA cells (Cat. No. K1770)

Item	Source
Equipment	
Fluorescence plate reader with bottom-read capabilities	MLS
Filters (if required for the plate reader)	Chroma Technologies
(Optional) Mr. Frosty™ Freezing Container	5100-0001
(Optional) Epifluorescence- or fluorescence-equipped microscope (with appropriate filters)	MLS
(Optional) Microplate centrifuge	MLS
Consumables	
Black-wall, clear-bottom, 384-well assay plates (with low fluorescence background)	Corning 3764
Compressed air	MLS
Reagents	
FreeStyle™ 293 Expression Medium	12338018
DMSO (for compound preparation)	MP Biomedical 196055
AR231453	Custom
Penicillin-Streptomycin (10,000 U/mL) (antibiotics)	15140122
Zeocin™ Selection Reagent	R25001
Geneticin™ Selective Antibiotic (G418 Sulfate) (50 mg/mL)	10131027
Hygromycin-B	10687010

Table 5 Additional materials required for dividing cells (Cat. No. K1777)

Item	Source
Reagents	
LiveBLAzer™-FRET B/G Loading Kit: • LiveBLAzer™-FRET B/G Substrate (CCF4-AM) (Cat. No. K1089)[1] • DMSO for Solution A • Solution B • Solution C	K1095[2]
Solution D	K1156
Recovery™ Cell Culture Freezing Medium	12648010
McCoy's 5A (Modified) Medium	16600082
Fetal bovine serum (FBS), dialyzed, tissue-culture grade **(Do Not Substitute.)**	26400036
MEM Non-Essential Amino Acids Solution (100X)	11140050
Sodium Pyruvate (100 mM)	11360070
HEPES (1 M, pH 7.3)	15630080
DPBS, no calcium, no magnesium	14190136
0.05% Trypsin-EDTA	25300054
Penicillin-Streptomycin (10,000 U/mL) (antibiotics)	15140122
Zeocin™ Selection Reagent	R25001
Geneticin™ Selective Antibiotic (G418 Sulfate) (50 mg/mL)	10131027
Hygromycin-B	10687010

[1] Cat. No. K1089 can be purchased separately from the kit.
[2] Additional kit sizes are available.

Cell culture conditions

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

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

Media requirements

Unless otherwise indicated, equilibrate all media and solutions to 37°C (recommended) or room temperature before adding to the cells.

IMPORTANT! Do not make media substitutions. The cell lines have been validated for optimal assay performance using the media indicated. For dividing cells, we recommend storing an aliquot of cells for back-up in case of contamination or loss of cell supply.

Component	DA and dividing cells	Dividing cells only		
	Assay Medium	Growth Medium	Thawing Medium	Freezing Medium
FreeStyle™ 293 Expression Medium	100%	—	—	—
McCoy's 5A (Modified) 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 (100 mM)	—	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™ Selection Reagent	—	200 µg/mL	—	—
Hygromycin-B	—	50 µg/mL	—	—
Geneticin™ antibiotic	—	100 µg/mL	—	—

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 “Assay procedure” on page 8, 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 × 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 (see “Assay procedure” on page 8).

   For guidance on using cells in an assay, proceed to “Assay procedure” on page 8.

Dividing cells

Thawing method

Note: The cells are shipped on dry ice and may require a short recovery period before 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 the medium to equilibrate to the proper pH and temperature.
3. Remove the vial of cells to be thawed from liquid nitrogen, then rapidly thaw by placing the vial in a 37°C water bath with gentle agitation for 1–2 minutes. Do not submerge the vial in water.
4. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
5. Transfer the vial contents, drop-wise, into 10 mL of Thawing Medium in a sterile 15-mL conical tube.
6. Centrifuge the cells at 200 × *g* for 5 minutes.
7. Aspirate the supernatant, then resuspend the cell pellet in 1 mL of fresh Thawing Medium.
8. Transfer the contents to the T75 tissue culture flask containing pre-equilibrated Thawing Medium, then place the flask in the humidified 37°C/5% CO_2 incubator.
9. At first passage, switch to Growth Medium.

Propagation method

- Passage or feed cells at least twice a week.
- Maintain cells between 25% and 95% confluence. Do not allow cells to reach confluence.

1. 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), then swirl to coat the cells evenly. The cells usually detach after approximately 2–5 minutes exposure to 0.05% Trypsin-EDTA.
2. 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 the cells at 200 × *g* for 5 minutes, then resuspend in Growth Medium.

Freezing method

1. Harvest the cells as described (see “Propagation method” on page 7). After detachment, count the cells, centrifuge at 200 × *g* for 5 minutes, then resuspend the cells in 4°C Freeze Medium at a density of 2×10^6 cells/mL.
2. Transfer 1.0-mL aliquots into cryogenic vials.
3. Place in an insulated container for slow cooling (we recommend Mr. Frosty™ Freezing Container, Cat. No. 5100-0001), then store overnight at -80°C.
4. Transfer to liquid nitrogen the next day for long-term storage.

Assay procedure

The following instructions outline the recommended procedure for determining activity of compounds as modulators of GPR119 using LiveBLAzer™-FRET B/G Substrate as the readout. If alternative substrates are used (for example, ToxBLAzer™ DualScreen or LyticBLAzer™ Loading kits), follow the loading protocol provided with the product.

Procedural guidelines

- Work on a dust-free, clean surface.
- Do not touch the bottom of the 384-well, black-wall, clear-bottom assay plate. Always handle the assay plate by the sides.
- If pipetting manually, briefly centrifuge the plate at room temperature (at 14 × *g* for 1 minute) after adding all assay components to ensure the reagents are on the bottom of the wells.
- 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.
- Some solvents may affect assay performance. Assess the effects of solvents before screening. The cell stimulation procedure described in the following sections is carried out in the presence of 0.1% DMSO to simulate the effect that a Test Compound's solvent can have on the assay. If other solvents and/or solvent concentrations are used, optimize the assay conditions appropriately.

Quick assay reference guides

For a more detailed assay protocol, see the next section.

Table 6 Agonist Assay Quick Reference Guide

	Unstimulated Control wells	Stimulated Control wells	Cell-Free Control 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 at 37°C/ 5%CO_2 for 0 hours.			
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 with 0.5% DMSO
Step 3: Incubate cells	Incubate in a humidified 37°C/5% CO_2 incubator for 16 hours.			
Step 4: Prepare 6X Substrate Mixture	1. Combine 6 µL of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) (Solution A) + 60 µL of Solution B, then vortex. 2. Add 904 µL of Solution C, then vortex. 3. Add 30 µL of Solution D, then vortex.			
Step 5: Add Substrate Mixture	Add 8 µL per well.			
Step 6: Incubate Substrate Mixture with cells	Incubate at room temperature for 2 hours in the dark.			
Step 7: Detect activity	See "Detection" on page 11.			
Step 8: Analyze data	See "Data analysis" on page 11.			

Table 7 Antagonist Assay Quick Reference Guide

	Unstimulated Control wells	Stimulated Control wells	Antagonist Control wells	Cell-Free Control 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 at 37°C/ 5%CO_2 for 0 hours.				
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 16 hours.				
Step 5: Prepare 6X Substrate Mixture	1. Combine 6 µL of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) (Solution A) + 60 µL of Solution B, then vortex. 2. Add 904 µL of Solution C, then vortex. 3. Add 30 µL of Solution D, then vortex.				
Step 6: Add Substrate Mixture	Add 8 µL per well.				
Step 7: Incubate Substrate Mixture with cells	Incubate at room temperature for 2 hours in the dark.				
Step 8: Detect activity	See “Detection” on page 11.				
Step 9: Analyze data	See “Data analysis” on page 11.				

Detailed assay protocol

Plate cells

1. Thaw DA cells/harvest dividing cells, then resuspend in Assay Medium to a density of 312,500 cells/mL.
2. Add 32 µL per well of 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.
3. Incubate cells at 37°C/ 5% CO_2 for 0 hours.
4. Proceed to the appropriate assay procedure.
   - For the Agonist assay—see “Agonist assay plate setup” on page 9.
   - For the Antagonist assay—see “Antagonist assay plate setup” on page 10.

Agonist assay plate setup

This section provides directions for performing an Agonist assay. See the next section 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.

   Note: We recommend running a dose response curve to determine the optimal concentration of the Agonist solution.
4. Add 8 µL of Assay Medium with 0.5% DMSO (prepared in step 1) to the Unstimulated Control and Cell-Free Control wells.
5. Add 8 µL of the 5X Agonist stock solution to the Stimulated Control wells.

6. Add 8 µL of the 5X Test Compound stock solution to the Test Compound wells.

7. Incubate the Agonist assay plate in a humidified 37°C/5% CO_2 incubator for 16 hours. Then proceed to "Substrate preparation, loading, and incubation" on page 10.

Antagonist assay plate setup

This section provides directions for performing an Antagonist assay. See the previous section 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.

   Note: 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.

   Note: We recommend running a dose response curve to determine the optimal inhibition concentration for the Antagonist solution.

5. Add 4 µL of the 10X Test Compound stock solution 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 Antagonist stock solution in Assay Medium with 0.5% DMSO to the Antagonist Control wells.

8. *(Optional)* If desired, incubate the Test Compounds with the cells in a humidified 37°C/5% CO_2 incubator before proceeding. A 30-minute incubation is typically sufficient.

9. Add 4 µL of the 10X Agonist stock solution 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 (prepared in step 1) 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 hours. Then proceed to "Substrate preparation, loading, and incubation" on page 10.

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.

IMPORTANT! 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 aliquots of the stock solution at –20°C until use.

   Note: The molecular weight of the LiveBLAzer™-FRET B/G Substrate (CCF4-AM) is 1096 g/mol.

2. Prepare 6X Substrate Mixture.
   a. Add 6 µL of Solution A to 60 µL of Solution B, then vortex.
   b. Add 904 µL of Solution C to the above solution, then vortex.
   c. Add 30 µL of Solution D to the above solution, then vortex.

3. Remove the assay plate from the humidified 37°C/5% CO_2 incubator.

   Note: Handle the plate gently. Do not touch the bottom of the assay plate.

4. Add 8 µL of 6X Substrate Mixture to each well.

5. Cover the plate to protect it from light and prevent evaporation.
6. Incubate at room temperature for 2 hours.

Detection

Measure fluorescence at room temperature from the bottom of the wells.

Before reading the plate, use compressed air to remove any dust from the bottom of the plate.

Instrumentation, filters, and plates

- Use a fluorescence plate reader with bottom-reading capabilities.
- Use the following recommended filters for the fluorescence plate reader:
  - Excitation filter: 409/20 nm
  - Emission filter: 460/40 nm
  - Emission filter: 530/30 nm

Read 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[1]	Scan 2[2]
Excitation filter	409/20 nm	409/20 nm
Emission filter	460/40 nm	530/30 nm

[1] Measures fluorescence in the Blue channel.
[2] Measures FRET signal in the Green channel.

Data analysis

Subtract the background, then calculate the emission ratio

We recommend subtracting 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.

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 can 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.

The following recommended filter set for observing beta-lactamase activity is available from Chroma Technology (**www.chroma.com**).

Chroma Complete Filter 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).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

Zlokarnik G, Negulescu PA, Knapp TE, Mere L, Burres N, Feng L, Whitney M, Roemer K, Tsien RY (1998) Quantitation of Transcription and Clonal Selection of Single Living Cells with Beta-Lactamase as Reporter. *Science* 279:84–88.

Kunapuli P, Ransom R, Murphy K, Pettibone D, Kerby J, Grimwood S, Zuck P, Hodder P, Lacson R, Hoffman I, Inglese J, Strulovici B (2003) Development of an Intact Cell Reporter Gene Beta-lactamase Assay for G Protein-coupled Receptors. *Analytical Biochem* 314:16–29.

Xing H, Tran HC, Knapp TE, Negulescu PA, Pollok BA (2000) A Fluorescent Reporter Assay For The Detection of Ligands Acting Through G1 Protein-coupled Receptors. *J Recept Signal Transduct Res* 20:189–210.

Qureshi SA, Sanders P, Zeh K, Whitney M, Pollok B, Desai R, Whitney P, Robers M, Hayes SA (2003) A One-Arm Homologous Recombination Approach for Developing Nuclear Receptor Assays in Somatic Cells. *Assay Drug Dev Technol* 1:755–766.

Peekhaus NT, Ferrer M, Chang T, Kornienko O, Schneeweis JE, Smith TS, Hoffman I, Mitnaul LJ, Chin J, Fischer PA, Blizzard TA, Birzin ET, Chan W, Inglese J, Strulovici B, Rohrer SP, Schaeffer JM (2003) A beta-lactamase-dependent Gal4-estrogen receptor beta transactivation assay for the ultra-high throughput screening of estrogen receptor beta agonists in a 3456-well format. *Assay Drug Dev Technol* 1:789–800.

Chin J, Adams AD, Bouffard A, Green A, Lacson RG, Smith T, Fischer PA, Menke JG, Sparrow CP, Mitnaul LJ (2003) Miniaturization of Cell-Based, Beta-Lactamase-Dependent FRET Assays to Ultra-High Throughput Formats to Identify Agonists of Human Liver X Receptors. *Assay Drug Dev Technol* 1:777–787.

Whitney M, Rockenstein E, Cantin G, Knapp T, Zlokarnik G, Sanders P, Durick K, Craig FF, Negulescu PA (1998) A Genome-wide Functional Assay of Signal Transduction in Living Mammalian Cells. *Nat Biotechnol* 16:1329–1333.

Fursov N, Cong M, Federici M, Platchek M, Haytko P, Tacke R, Livelli T, Zhong Z (2005) Improving Consistency of cell-based assays by using division-arrested cells. *Assay Drug Dev Technol* 3:7–15.

Kunapuli P, Zheng W, Weber M, Solly K, Mull R, Platchek M, Cong M, Zhong Z, Strulovici B (2005) Application of division arrest technology to cell-based HTS: comparison with frozen and fresh cells. *Assay Drug Dev Technol* 3:17–26.

Digan ME, Pou C, Niu H, Zhang JH (2005) Evaluation of division-arrested cells for cell-based high-throughput screening and profiling. *J Biomol Screen* 10:615–623.

Vasudevan C, Fursov N, Maunder P, Cong M, Federici M, Haskins JR, Livelli T, Zhong Z (2005) Improving high-content-screening assay performance by using division-arrested cells. *Assay Drug Dev Technol* 3:515–523.

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.