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User Manual

ChemiSCREEN™ and ChemiBRITE™ Cell Lines

For Fluorescent or Chemiluminescent Detection of GPCR-Mediated Intracellular Calcium Mobilization

Document Number 70-427 Revision 1

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Table of Contents

Important: Please read this entire user manual before proceeding with the assay.

Overview ... 1
ChemiSCREEN Cell Lines ... 1
ChemiBRITE Cell Lines ... 1
Materials Provided ... 2
Storage Conditions ... 2
Additional Materials Required ... 2
Unpacking Cell Cryovials ... 3
Cell Culture Protocol ... 4
Cell Thawing ... 4
Cell Recovery ... 4
Cell Propagation ... 5
Cell Freezing ... 6
Detailed Assay Protocol: Agonist and Antagonist ... 8
1. Preparation and Plating of Cells ... 8
2. Dye Loading for Fluorescent Calcium Flux Assays ... 9
3. Dye Loading for Luminescent Calcium Flux Assays (For ChemiBRITE Cell Lines) ... 10
4. Compound Preparation ... 11
5. Detection for Fluorescent and Luminescent Calcium Flux Assays ... 12
6. Data Reduction ... 14
Typical Results ... 14
Appendix 1: Cell Culture Media Requirements ... 15
1. ChemiSCREEN Cell Lines ... 15
2. ChemiBRITE Cell Lines ... 16
Frequently Asked Questions ... 18
Limited Use License Agreement ... 20
Contact Information ... 20

For additional information or Technical Support, see contact information at the bottom of this page.

Overview

The ChemiSCREEN and ChemiBRITE Cell Lines are designed to enable the measurement of intracellular calcium levels when a GPCR of interest has been activated by a ligand. These cell lines are suitable for studying small molecules as well as biologics. The assay protocol has been optimized for use in either a 384-well or 96-well format.

ChemiSCREEN and ChemiBRITE cells stably express the target of interest in cell backgrounds that are highly suitable for measurement of calcium flux upon receptor activation. Depending on the target, the cells can also contain recombinant promiscuous G proteins to allow for coupling to the calcium signaling pathway. This approach provides a common platform for interrogating receptor signaling independently of the native coupling status.

ChemiSCREEN Cell Lines

ChemiSCREEN cell lines have been qualified for use with calcium-sensitive fluorescent dyes that measure calcium flux via release of calcium from intracellular stores.

ChemiBRITE Cell Lines

ChemiBRITE cell lines also express a novel variant of clytin, a calcium-activated photoprotein, to enable sensitive luminescent detection of ligand-induced calcium flux. The ChemiBRITE version of clytin contains a mutation that increases its affinity for calcium to a level that permits detection of cytosolic calcium in many cells with greater sensitivity than other photoproteins targeted to the mitochondria. Luminescent calcium assays offer several advantages over fluorescent calcium assays including increased sensitivity and lack of interference from fluorescent compounds. The cell lines have been qualified for use with both luminescent calcium flux detection using coelentrazine as a substrate, as well as with calcium sensitive fluorescent dyes. This enhanced capability increases the range of instruments that can be used to measure calcium signaling.

Materials Provided

Components	
2 vials of cells	Refer to cell line-specific datasheet for shipped cell density

Storage Conditions

Cells must arrive in a frozen state on dry ice, and should be transferred to the vapor-phase of liquid nitrogen storage or a -80°C freezer immediately upon arrival. If cells will be thawed and used within 24 hours, they can be stored temporarily at -80°C. For long-term storage, store vials in the vapor phase of liquid nitrogen.

Contact technical support immediately if cells received were already thawed.

Additional Materials Required

Refer to the table below to determine the appropriate media and reagents required for the specific cell line used in the assay. The cell type is provided in the cell line-specific datasheet.

Material	**Ordering Information** **Recommended Products (or Equivalent)**
Hanks' Balanced Salt Solution (HBSS)	HyClone, Cat. No. SH3026802, or Invitrogen, Cat. No. 14025
HEPES 1M Stock	Millipore Sigma, Cat. No. H3375, or TMS-003-C
Probenecid	Millipore Sigma, Cat. No. P8761
BSA (Protease-free); prepared to 1% in water, filtered	Millipore Sigma, Cat. No.126609
Fluo-8® AM	AAT Bioquest, Cat. No. 21080
Non-binding 96-well plates (for ligand preparation)	Eurofins DiscoverX, Cat. No. 92-0011
Black, clear-bottom, tissue culture treated plates	Corning, Cat. No. 3904/3712
FLIPR tips or equivalent	Refer to the recommendations provided by the instrument's manufacturer
Ligands	Refer to the cell line-specific datasheet
Cell culture (growth) media	Refer to the tables in Appendix 1 for cell line-specific media requirements
Selection antibiotics	Refer to the tables in Appendix 1

Unpacking Cell Cryovials

Two cryovials are shipped on dry ice, and each contain cells in 1 mL of Freezing Reagent (refer to the cell line-specific datasheet for the number of cells provided in the vials). The following procedures are for safe storage, handling, and removal of cryovials from the vapor phase of liquid nitrogen storage.

1. Cells must arrive in a frozen state on dry ice.

Contact technical support immediately if cells received were already thawed.

2. Frozen cells must be transferred to either the vapor phase of liquid nitrogen or a -80°C freezer immediately upon arrival. If cells will be thawed and used within 24 hours, they can be stored temporarily at -80°C. For long-term storage, cryovials must be stored in the vapor-phase of liquid nitrogen.

Cryovials should be stored in the vapor phase of liquid nitrogen. They are not rated for storage in the liquid phase.

3. Using tongs, remove the cryovials from the liquid nitrogen storage, and place them immediately in dry ice, in a covered container.

A face shield, gloves, and a lab coat should be worn during these procedures.

4. Wait for at least one minute for any liquid nitrogen that may be present inside the vial to evaporate.

5. Proceed with the thawing and propagation protocol in the following section. Refer to the cell line-specific datasheet for appropriate products mentioned in the following protocols.

Cell Culture Protocol

The following procedures are for thawing adherent cells from cryovials, seeding and expanding the cells, and freezing the cells once they have been propagated. Refer to the cell-line specific datasheet to identify the cell type of the cell line. Refer to Appendix 1 to determine the correct growth media formulation for the specific cell type.

For initial cell thawing steps and passage 1, selection antibiotics should not be added to the growth media.

Cell Thawing

The following is a protocol for thawing cells in a T75 flask.

1. Pre-warm the growth media (without antibiotics) in a 37°C water bath for 15 minutes, as indicated on the cell line-specific datasheet.

2. Add 15 mL of the growth media into a T75 flask inside a sterile tissue culture hood. Set aside for Step 6. DO NOT add selection antibiotics to the growth media at this time/stage.

3. Remove the cryovials from -80°C or liquid nitrogen vapor storage and immediately place them in dry ice prior to thawing.

Safety Warning: A face shield, gloves, and a lab coat should be worn at all times when handling frozen vials. When removing cryovials from the vapor phase of liquid nitrogen storage, use tongs to place them immediately in dry ice in a covered container. Wait at least 1 minute for any liquid nitrogen inside the vial to evaporate. Do not touch the bottom of the vials at any time, to avoid inadvertent thawing of the cells.

4. Place the cryovials containing the cells in a 37°C water bath briefly (30 seconds to 1 minute), until only small ice crystals remain and the cell pellet is almost completely thawed. DO NOT centrifuge or vortex freshly thawed cells.

Do not leave the frozen cell vials in the water bath for longer than 1 minute. Prolonged thawing at 37ºC may result in cell death.

5. Decontaminate the external surface of the vials by spraying and wiping them with 70% ethanol. Transfer the vials to a tissue culture hood.

6. Using a sterile pipette, gently transfer the thawed cells to the pre-filled T75 flask. Incubate the flask at 37°C and 5% CO_2.

7. Maintain the cells in culture until they are 70-80% confluent. Then, proceed to Cell Recovery instructions. Do not split if cells are below this confluency, or growth issues may occur.

Cell Recovery

The following is a protocol for ensuring maximal cell recovery once they become 70-80% confluent in a T75 flask.

1. Pre-warm the growth media in a 37°C water bath for 15 minutes. DO NOT add selection antibiotics to the growth media.

2. Remove the T75 flask from the tissue culture incubator and place it in a sterile tissue culture hood.

3. Gently aspirate the media from the T75 flask.

4. Add 5 mL of PBS into the T75 flask, and gently rock the flask back and forth to rinse the cells.

5. Gently aspirate PBS from the flask.

6. Add 1 mL of 0.25% Trypsin-EDTA to the flask. Gently rock the flask back and forth to ensure that the inner surface of the flask is uniformly covered with trypsin.

7. Incubate the flask at 37°C and 5% CO_2 for 2 to 3 minutes, or until the cells have detached.

8. Remove the flask from the incubator and observe the cells under a microscope to confirm cell detachment. If necessary, gently tap the edge of the flask to detach cells from the surface. If most of the cells have not yet detached, then return the flask to the incubator for additional 1 to 2 minutes. Repeat this step until all cells are in suspension.

Some cell lines are highly adherent and therefore may require additional dissociation time.

9. Using a pipette, slowly add 4 mL of the growth media to the detached cells in T75 flask. Using a pipette, gently rinse the cells with the reagent. This will also neutralize the trypsin in the cell suspension. .

10. Gently pipet the cell suspension up and down several times to generate a single cell suspension without any clumps.

11. Count the number of viable cells using a hemocytometer or an automated cell counter. Determine the number of cells and cell suspension volume required to seed into a T75 or T225 flask. Cells should be seeded according to the planned growth period. Refer to the table below for cell seeding recommendations.

Flask Size	Volume (mL)	Total Cell Number (x 10^6)	Growth Period (h)
T75	15	4.0 - 5.0	24
T75	15	2.0	48
T75	15	0.5	72

12. Add 5 mL of growth media to a new T75 or T225 flask, followed by addition of the cell suspension (volume determined in Step 11). Add an additional volume of the growth media to reach a final volume of 15 mL for a T75 flask, or 45 mL for a T225 flask.

13. Transfer the flask to a tissue culture incubator and incubate the cells at 37°C and 5% CO_2.

Cell Propagation

The cells can be propagated after a successful recovery, which can be determined by monitoring them under a microscope. Healthy cells should adhere uniformly to the surface of the flask, with only a few cells remaining in suspension.

To maintain logarithmic growth of the cells, cultures should be maintained in a sub-confluent monolayer.

1. If the cells appear healthy:

    1.1. Exchange the growth media with cell culture media; 15 mL for a T75 flask or 45 mL for T225 flasks), supplemented with the recommended concentration of selection antibiotic. Refer to Appendix 1 to determine the correct growth media, recommended selection antibiotic, and antibiotic concentration for the cell line.

2. If the cells do not appear to be healthy, or if confluency is <25%:

    2.1. Incubate the flask for additional 24 to 48 hours to allow for cell recovery before adding the cell culture media.

3. Once the cells have reached 70-80% confluency, split the cells every 2 to 3 days, based on the doubling time of the cell line. Use the cell culture media supplemented with the appropriate selection antibiotics (as indicated in Appendix 1) to split the cells.

Cell Freezing

The following procedure is for freezing cells that have been propagated in T75 or T225 flasks. This protocol assumes that the cells have reached 70-80% confluency in a sufficient number of flasks to fill the desired number of cryovials. The cells will be cryopreserved in aliquots of 1 mL/vial, at the desired number of cells per vial (e.g. 2 x 10^6 per vial).

1. Remove T75 (or T225) flasks from the incubator and place them in a sterile tissue culture hood.

Care should be taken while handling flasks to avoid contamination.

2. Slowly aspirate the media from the flasks.
3. Add 10 mL of PBS into each T75 flask (or 15 mL for a T225 flask), and gently rock the flask back and forth to ensure that the cells are rinsed.
4. Gently aspirate PBS from the flask.
5. Add 1 mL of 0.25% Trypsin-EDTA to T75 flasks (or 3 mL to T225 flasks).
6. Gently rock the flask back and forth to ensure that the inner surface of the flask is uniformly covered with Trypsin-EDTA.
7. Incubate the flasks at 37°C and 5% CO_2 for 2 to 3 minutes, or until the cells have detached.
8. Remove the flask from the incubator and view under a microscope to confirm that the cells have detached. If necessary, gently tap the edge of the flask to detach cells from the surface. If most of the cells have not detached then return the flask to the incubator for additional 1 to 2 minutes and repeat this step until all cells are in suspension.
9. Add 5 mL of the cell culture media to each T75 flask (or 15 mL to each T225 flask) to neutralize the trypsin.
10. Gently pipet the cell suspension up and down several times to generate a single cell suspension without any clumps.

11. Transfer the cell suspension from the T75 flask into a 15 mL conical centrifuge tube. If using a T225 flask, transfer cells to a 50 mL conical centrifuge tube. If necessary, add an additional volume of the cell culture media to the flasks (e.g. 3 mL for T75 flasks or 5 mL for T225 flasks). Rinse the flask to collect the remaining cells, then transfer it into the conical tube. Slowly pipette up and down several times to break up clumps of cells.

12. To determine the concentration of cells in the suspension:

    12.1. Set aside a small fraction (0.5 mL or less) of the suspended cells in a separate tube.

    12.2. Transfer an appropriate portion of this fraction to a hemocytometer (typically 10 µL of cell suspension) or another cell counting device.

    12.3. Count cells, calculate the concentration of cells in the suspension, and then calculate the total number of cells in the 15 mL (or 50 mL) centrifuge tube.

13. Centrifuge the collected cells at 200 x g for 4 minutes.

14. After centrifugation, discard the supernatant, being careful not to disturb the cell pellet.

15. Based on the total cell number calculated in Step 12, resuspend the cells to the desired concentration (e.g. 2×10^6 cells/mL) with ice-cold CryoMedium. Refer to the table below for the ratio of components used to prepare the CryoMedium:

Growth Media (Without Selection Antibiotics)	40%
Fetal Bovine Serum (FBS)	50%
Dimethyl Sulfoxide (DMSO)	10%

Keep cells on ice during this process to protect cell viability.

16. Aliquot 1 mL of the cell suspension into each of the labeled 2 mL cryogenic tubes. Seal the tubes tightly.

17. Freeze cells in a -80°C freezer at a controlled rate of -1°C/minute overnight in a dedicated cell freezer or commercially-available freezing chamber (pre-chilled to 4°C). For short-term storage, vials can be stored in the -80°C freezer for a maximum of two weeks.

18. Transfer the vials into the vapor phase of liquid nitrogen for long-term storage.

Detailed Assay Protocol: Agonist and Antagonist

The following protocol is for evaluating the response with an agonist or antagonist in a 384-well assay plate. Refer to the table below for reagent volumes for either 96- or 384-well assays.

Assay Reagents (Volume per Well)	**96-Well Plate***		**384-Well Plate****	
	Agonist Assay	Antagonist Assay	Agonist Assay	Antagonist Assay
Cell Plating Volume* (µL)	100	100	25	25
Dye Loading Buffer (µL)	160	160	40	40
Primary Addition: Agonist Assay (µL)	80	80	20	20
Secondary Addition: Antagonist Assay (µL)	-	80	-	20
Total Assay Volume (µL)	**240**	**320**	**60**	**80**

*The cell plating volume refers to the growth media used while plating the cells. The media is removed and replaced with the dye loading buffer at the start of the assay set-up.

1. Preparation and Plating of Cells

The following protocol provides steps for harvesting and preparing adherent cells for plating in an assay plate. This protocol assumes that cells have reached a 70-80% confluency and have been cultured in the recommended cell culture media. For cell line-specific information on the control ligand, incubation times and temperature, please refer to the cell line-specific datasheet. Cells are seeded into assay plates using the same growth media used for culturing cells but with the selection antibiotics excluded (refer to Appendix 1 for details).

1.1. Ensure that the cells are in the logarithmic growth phase prior to use in the assay.

1.2. Warm the cell culture media without selection media in a clean 37°C water bath for 15 minutes. Refer to Appendix 1 for the appropriate media for the cell type).

1.3. Dissociate the cells and resuspend them in fresh media.

1.3.1. Aspirate the media from the T75 flasks. Add 10 mL of room temperature PBS into each flask, and gently swirl it to rinse the cells. Aspirate PBS from the flask.

Use calcium- and magnesium-free PBS for rinsing cells prior to detachment.

1.3.2. Dissociate the cells by adding 1 mL of 0.25% trypsin-EDTA to the T75 flasks (or 3 mL for T225 flasks). Gently rock the flask back and forth to ensure that the surface of the flask is uniformly covered.

1.3.3. Incubate the flask at 37°C and 5% CO_2 in a humidified incubator for 2-3 minutes, or until the cells have detached.

1.3.4. Remove the flask from the incubator and confirm that the cells have detached by viewing under a microscope. Gently tap the edge of the flask to detach cells from the inner surface, if necessary.

1.3.5. Add 8-10 mL of the growth media to each flask and pipette up and down a few times to dissociate cells.

1.4. Remove an aliquot of the cells from the flask and determine the cell density (e.g. using a hemocytometer or automated cell counter).

1.5. Transfer an appropriate volume of the cell suspension to a conical tube, depending on the number of cells required for the desired number of samples to be run in the assay. Centrifuge at 300 x g for 4 minutes at room temperature to pellet cells.

1.6. Decant the supernatant or carefully remove the media with a 10 mL pipette, without disturbing the cell pellet.

1.7. Resuspend the cell pellet in the recommended growth media. Refer to the cell line-specific datasheet for the recommended concentration of cells. Based on the number of cells in the pellet from Step 1.5, resuspend the pellet in an appropriate volume to achieve the desired concentration of cells (e.g. 500,000 cells/mL or 12,500 cells/25 µL).

1.8. Pour the cell suspension into a sterile reagent reservoir. Transfer 25 µL of the cell suspension into each well of a 384-well assay plate using a multichannel pipette.

1.9. Incubate the assay plate at 37°C and 5% CO_2 before proceeding with the agonist antibody preparation.

2. Dye Loading for Fluorescent Calcium Flux Assays

The following protocol is for the fluorescent detection of calcium flux, for both ChemiSCREEN and ChemiBRITE cell lines. To detect calcium flux using luminescence (ChemiBRITE cell lines only), refer to section 3. Dye Loading for Luminescent Calcium Flux Assays.

2.1. The following table lists various solutions and their individual components that are required for dye loading in fluorescent calcium flux assays.

Solution Name	Components	Concentration (mM)	Formula Weight	Amount	Storage
Assay Buffer	1X HBSS	N/A	N/A	980 mL	Up to 3 months at 4°C
	1 M HEPES	20	238.3	20 mL	
Dye Buffer	1X HBSS	N/A	N/A	980 mL	
	1 M HEPES	20 mM	238.3	20 mL	
	Probenecid	2.5 mM	285.36	710 mg	
Calcium Dye	Fluo-8 AM dye	5 mM	1046.93	1 mg	Up to 6 months at - 20°C
	DMSO	N/A	N/A	200 µL	

2.2. Ensure that any premade buffers are brought to room temperature prior to use.

2.3. Add 20 µL of Calcium Dye to 20 mL of Dye Buffer. This volume is sufficient for a single 384-well plate.

2.4. Remove the media from the plate. An example method of removing the media would be to hold the plate by the edges (without touching the bottom of the plate), and inverting it quickly to flick out the media from the wells.

2.5. Without turning the plate over, blot the plate on a paper towel to remove any residual media.

2.6. Using a multi-channel pipette or reagent dispenser, load 40 µL of dye to each well.

2.7. Place the plate in a 30ºC (± 2°C) incubator.

2.7.1. Cells can be incubated at 37°C, however, incubating at the 30ºC (± 2°C) will prevent continued cell division before running the calcium flux assay.

2.7.2. Cells should be used in the assay between 60-90 minutes after dye loading.

3. Dye Loading for Luminescent Calcium Flux Assays (For ChemiBRITE Cell Lines)

The following protocol is for the luminescent detection of calcium flux for ChemiBRITE cell lines. To detect calcium flux using fluorescence (both ChemiSCREEN and ChemiBRITE cell lines), refer to section 2. Dye Loading for Fluorescent Calcium Flux Assays.

3.1. The following table lists various solutions and their individual components that are required for dye loading in luminescent calcium flux assays.

Solution Name	Components	Concentration	Formula Weight	Amount	Storage
Assay Buffer	1X HBSS	N/A	N/A	970 mL	Store at 4°C for up to 3 months
	1 M HEPES	20 mM	238.3	20 mL	
	10% BSA	10%	N/A	10 mL	
Loading Buffer	1X HBSS	N/A	N/A	970 mL	
	1 M HEPES	20 mM	238.3	20 mL	
	10% BSA	10%	N/A	10 mL	
	10 µM Coelenterazine-h	10 µM	423.46	710 mg	
10 mM Coelenterazine-h	Coelenterazine-h	N/A	423.46	250 µg	Refer to the manufacturer's recommendation
	Methanol	N/A	N/A	59 µL	

3.2. Ensure that any pre-made buffers are brought to room temperature prior to use.

3.3. Prepare the Loading Buffer: Add 20 µL of 10 mM Coelenterazine-h to 20 mL of Assay Buffer. This is sufficient volume for a single 384-well plate.

It is critical that Coelenterazine-h is prepared at room temperature and is protected from light.

The Coelenterazine-h stock should be prepared according to the manufacturer's recommendations at 10 mM to allow for 1:1000 dilution in the Loading Buffer (to a final concentration of 10 µM). Refer to manufacturer's recommendation for storing Coelenterazine-h.

3.4. Remove the media from the plate. One method of removing the media would be to hold the plate by the edges (without touching the bottom of the plate), and inverting it quickly to flick out the media from the wells.

3.5. While still inverted before turning the plate over, blot the plate on a paper towel to remove any residual media.

3.6. Using a multi-channel pipette or reagent dispenser, load 40 µL of dye to each well.

3.7. Place the plate in a 30°C (±2°C) incubator.

3.7.1. Cells can be incubated at 37°C, however incubating at the 30°C (±2°C) will prevent continued cell division before running the calcium flux assay.

3.7.2. Cells should be used in the assay between 2-3 hours after dye loading.

4. Compound Preparation

The following protocol is for preparing compounds for agonist or antagonist testing while cells are being loaded with dye. Samples are first evaluated for agonist activity by adding the compound to dye-loaded cells. Antagonist activity can be subsequently measured by challenging the same dye-loaded cells with an EC_{80} of a known agonist.

4.1. Compound preparation for agonist mode:

4.1.1. Prepare serial dilutions of the compound in Assay Buffer at 3X the desired final concentration in the assay. The serial dilutions can be performed in a 96-well plate and transferred to the four quadrants of a 384-well plate to generate four replica wells for each concentration.

4.1.2. Alternatively, compounds can be serially diluted at 100-1,000X the test concentration in DMSO and then subsequently diluted 33 to 333-fold in Assay Buffer to achieve 3X the desired final concentration (with 0.1% to 1% DMSO in the assay).

4.1.3. For dose-response curves using 10-11 concentrations, perform 3-fold serial dilutions.

4.1.4. For dose-response curves using 8-9 concentrations, perform 4-fold serial dilutions. This ensures that a top and bottom plateau is generated for the dose-response curve.

4.1.5. Include positive (ligand at maximal effective dose and vehicle) and negative controls (vehicle only) on the compound plate to define the maximal and minimal response, if required.

4.2. Compound preparation for antagonist mode:

4.2.1. Antagonist activity is typically measured in the presence of EC_{80} of agonist (ligand). To prepare the ligand at EC_{80}, dilute the ligand to 4X the final EC_{80} assay concentration in assay buffer. This can be prepared ahead of performing the agonist mode step if a fixed ligand challenge concentration is to be used.

4.2.2. If the EC_{80} concentration is to be determined empirically from the control ligand response generated in the agonist mode step, then the EC_{80} value should be calculated directly from the ligand dose-response curve after the agonist mode test is complete.

5. Detection for Fluorescent and Luminescent Calcium Flux Assays

5.1. Agonist activity:

5.1.1. Using the instrument's (e.g. FLIPR®) built-in dispenser, add 20 µL of the 3X compound to assay plate wells after 10 seconds of initiating the run.

5.1.2. Monitor the fluorescent signal over the course of 120-240 seconds.

5.2. Second addition to detect antagonist activity:

5.2.1. Using the instrument's built-in dispenser, add 20 µL of the 4X EC_{80} reagent assay plate wells after 10 seconds of initiating the run.

5.2.2. Monitor the fluorescent signal over the course of 120-240 seconds.

5.3. Recommended instrument settings:

Note: The following settings are provided as a general guideline for running these assays. Always refer to the instrument manufacturer's recommendations for instrument-specific settings.

5.3.1. FLIPR® Tetra with the EMD camera: Typically, time courses are between 120-240 seconds, with ligand addition at a 10 second time point.

Option	384-Well Plate
Read Mode	Fluorescence
Ex/Em	Ex470-495/Em515-575
Camera Gain	100
Gate Open	0%
Exposure Time	0.4 seconds
Read Interval	1 second
Dispense Volume	20 µL
Dispense Height	20 µL
Dispense Speed	30 µL/sec
Expel Volume	0 µL
Analysis	Max-Min

5.3.2. FLIPR Tetra with the ICCD camera: This machine can detect calcium flux using fluorescent and luminescent readouts. Typically, time courses are between 120-240 seconds, with ligand addition at a 10 second time point.

5.3.2.1. For fluorescent readouts:

Option	96-Well Plate	384-Well Plate
Read Mode	Fluorescence	Fluorescence
Ex/Em	Ex470-495/Em515-575	Ex470-495/Em515-575
Camera Gain	2,000	2,000
Gate Open	6%	6%
Exposure Time	0.53 seconds	0.53 seconds

Read Interval	1 second	1 second
Dispense Volume	50 µL	25 µL
Dispense Height	95 µL	50 µL
Dispense Speed	50 µL/sec	50 µL/sec
Expel Volume	0 µL	0 µL
Analysis	Subtract Bias Sample 1	Subtract Bias Sample 1

5.3.2.2. For luminescent readouts:

Option	96-Well Plate	384-Well Plate
Read Mode	Luminescence	Luminescence
Ex/Em	None/None	None/None
Camera Gain	280,000	280,000
Gate Open	100%	100%
Exposure Time	0.9 seconds	0.9 seconds
Read Interval	1 second	1 second
Dispense Volume	50 µL	25 µL
Dispense Height	95 µL	50 µL
Dispense Speed	50 µL/sec	50 µL/sec
Expel Volume	0 µL	0 µL
Analysis	Subtract Bias Sample 1	Subtract Bias Sample 1

5.3.3. FLIPR® Penta with the HS-EMDCC camera option: Typically, time courses are between 120-240 seconds, with ligand addition at a 10 second time point.

Option	96-Well Plate	384-Well Plate	1536-Well Plate
Read Mode	Fluorescence	Fluorescence	Fluorescence
Ex/Em	Ex470-495/ Em515-575	Ex470-495/ Em515-575	Ex470-495/ Em515-575
Camera Gain	4	6.5	4
LED Intensity	50%	50%	50%
Exposure Time	0.1 seconds	0.1 seconds	0.1 seconds
Read Interval	1 second	1 second	1 second
Dispense Volume	50 µL	12.5 µL	1 µL
Dispense Height	210-230 µL	30 µL	1 µL
Tip Up Speed	10 mm/sec	10 mm/sec	10 mm/sec
Addition Speed (Adherent cells)	50 µL/sec	30 µL/sec	8 µL/sec
Addition Speed (Non-Adherent cells)	20 µL/sec	20 µL/sec	2 µL/sec

6. Data Reduction

After the run is complete, apply subtract bias on sample 1, as indicated in Step 5.3 in the Detection for Fluorescent and Luminescent Calcium Flux Assays section. We recommend using Negative Control Correction with Buffer Only Wells. Export data according to research needs. For most Calcium Flux analysis, using Export of Max Signal to end of run is sufficient.

Typical Results

The following data are examples of responses obtained by the fluorescent and luminescent assays with the ChemiBRITE GLP-1 Glucagon Family Receptor Stable Cell Line

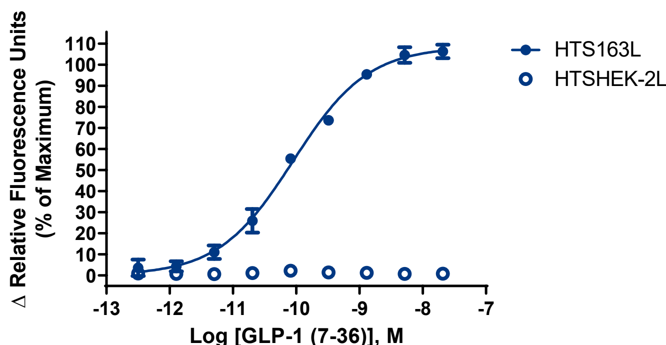

Figure 1. Typical Results (Fluorescence). Representative data for activation of GLP-1 receptor stably expressed in HEK293 cells induced by GLP-1(7-36) using a fluorescent calcium flux assay. GLP-1R–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate the following day the cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a FLIPR® Tetra with ICCD camera. Maximal fluorescence signal obtained in this experiment was 15,000 RLU. Similarly, parental cells (Cat. No. HTSHEK-2L) were tested to determine the specificity of the resulting signal.

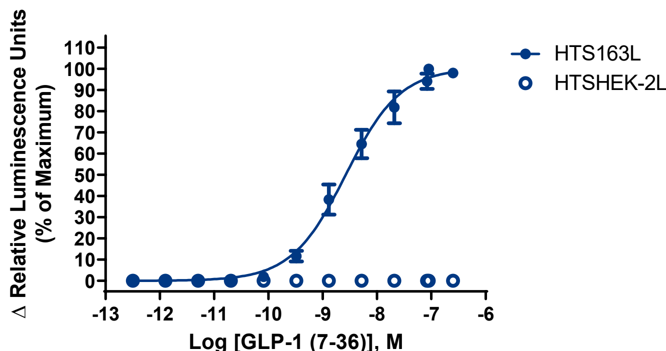

Figure 2. Typical Results (Luminescence). Representative data for activation of GLP-1 receptor stably expressed in HEK293 cells, induced by GLP-1(7-36) using a luminescent calcium flux assay. GLP-1R–expressing HEK293 cells were loaded with 10 µM Coelenterazine-h for 3 hours at room temperature and calcium flux in response to the indicated ligand was determined on a FLIPR Tetra with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 260,000 RLU (Relative Light Units) as measured by AUC (are under curve) for 80 seconds post agonist addition using the provided protocol. Similarly, parental cells (Cat. No. HTSHEK-2L) were tested to determine the specificity of the resulting signal.

Appendix 1: Cell Culture Media Requirements

Growth media used for each cell line background are listed in the following tables.

1. ChemiSCREEN Cell Lines

Cell Background	Media Component	Final Concentration	Supplier Name	Supplier Part Number
Chem-1	DMEM high glucose with 4mM L-glutamine; w/o sodium pyruvate	1X	Fisher Scientific	SH30022.LS
	Fetal Bovine Serum (FBS)	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	HEPES	10 mM	Millipore Sigma	H3537
	AssayComplete™ G418	250 µg/mL	Eurofins DiscoverX	92-0030
Chem-2	RPMI 1640	1X	Hyclone	SH30027.02
	Dialyzed FBS	20%	ThermoFisher	26400-044
	AssayComplete G418	250 µg/mL	Eurofins DiscoverX	92-0030
Chem-4 Chem-5 Chem-9 Chem-10	DMEM high glucose with 4mM L-glutamine; without sodium pyruvate	1X	Fisher Scientific	SH30022.LS
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	HEPES	10 mM	Millipore Sigma	H3537
	AssayComplete™ G418	250 µg/mL	Eurofins DiscoverX	92-0030
	AssayComplete™ Hygromycin B	250 µg/mL	Eurofins DiscoverX	92-0029
Chem-11	DMEM high glucose with 4mM L-glutamine; without sodium pyruvate	1X	Fisher Scientific	SH30022.LS
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	HEPES	10 mM	Millipore Sigma	H3537
	AssayComplete™ G418	250 µg/mL	Eurofins DiscoverX	92-0030
	Zeomycin	200 µg/mL	Gibco	R25001
Chem-6 Chem-7	F-12 Kaighn's	1X	ThermoFisher	21127-022
	FBS	10%	Millipore Sigma	F2442
	AssayComplete™ G418	250 µg/mL	Eurofins DiscoverX	92-0030
	Zeomycin	200 µg/mL	Gibco	R25001
Chem-8	F-12 Kaighn's	1X	ThermoFisher	21127-022
	FBS	10%	Millipore Sigma	F2442
	AssayComplete™ G418	250 µg/mL	Eurofins DiscoverX	92-0030

2. ChemiBRITE Cell Lines

Catalog Number	Component	Final Concentration	Supplier Name	Supplier Part Number
HTS048L HTS091L	DMEM/F12	1X	ThermoFisher	10565-042
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	HEPES	10 mM	Millipore Sigma	H3537
	AssayComplete™ Puromycin	1 µg/mL	Eurofins DiscoverX	92-0028
	AssayComplete™ G418	400 µg/mL	Eurofins DiscoverX	92-0030
	AssayComplete™ Hygromycin B	200 µg/mL	Eurofins DiscoverX	92-0029
HTS157L HTS185L HTS092L HTS238L HTS081L	DMEM/F12	1X	ThermoFisher	10565-042
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	AssayComplete™ Puromycin	1 µg/mL	Eurofins DiscoverX	92-0028
	AssayComplete™ G418	400 µg/mL	Eurofins DiscoverX	92-0030
	AssayComplete™ Hygromycin B	200 µg/mL	Eurofins DiscoverX	92-0029
HTS061L HTS099L HTS093L HTS038L HTS225L HTS131L	DMEM/F12	1X	ThermoFisher	10565-042
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	AssayComplete™ Puromycin	1 µg/mL	Eurofins DiscoverX	92-0028
	AssayComplete™ G418	400 µg/mL	Eurofins DiscoverX	92-0030
HTS178L	DMEM/F12	1X	ThermoFisher	10565-042
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	AssayComplete™ Puromycin	1 µg/mL	Eurofins DiscoverX	92-0028
	AssayComplete™ G418	300 µg/mL	Eurofins DiscoverX	92-0030
	AssayComplete™ Hygromycin B	150 µg/mL	Eurofins DiscoverX	92-0029

ChemiBRITE Cell Lines (Continued)

Catalog Number	Component	Final Concentration	Vendor	Part Number
HTS003L HTS163L HTS233L	DMEM/F12	1X	ThermoFisher	10565-042
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	AssayComplete™ Puromycin	1 µg/mL	Eurofins DiscoverX	92-0028
	AssayComplete™ G418	200 µg/mL	Eurofins DiscoverX	92-0030
	AssayComplete™ Hygromycin B	100 µg/mL	Eurofins DiscoverX	92-0029
HTSHEK-1L HTSU20S-1L	DMEM/F12	1X	ThermoFisher	10565-042
	FBS	1X	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	AssayComplete™ Puromycin	1 µg/mL	Eurofins DiscoverX	92-0028
HTSHEK-2L HTSHEK-3L HTSHEK-4L HTSHEK-5L HTSHEK-6L	DMEM/F12	1X	ThermoFisher	10565-042
	FBS	10%	Millipore Sigma	F2442
	Non-essential amino acids (NEAA)	1X	ThermoFisher	11140-050
	AssayComplete™ Puromycin	1 µg/mL	Eurofins DiscoverX	92-0028
	AssayComplete™ Hygromycin B	200 µg/mL	Eurofins DiscoverX	92-0029

Frequently Asked Questions

1. How do you determine EC_{80} from the agonist reference curve?

- Use an online EC_{80} calculator such as QuickCal by GraphPad, or
- Use the formula below, where F is the percent response and H is the HillSlope from the agonist reference curve

$$EC_F = \left(\frac{F}{100 - F}\right)^{\frac{1}{H}} EC_{50}$$

$$EC_{80} = \left(\frac{80}{100 - 80}\right)^{\frac{1}{H}} EC_{50}$$

2. What if there is no or low signal?

- Visually inspect the cells before and after compound incubation in a clear bottom plate to ensure that they are healthy. Make sure proper cell plating procedure is followed to ensure assay performance.
- Make sure that the cell line-specific detection reagents were used, and were stored and prepared as indicated in the datasheet.
- Make sure the proper assay mode is used (agonist mode or antagonist mode).
- Make sure the plate reader is setup properly to read the fluorescent or luminescent signal correctly.
- Only black-walled plates may decrease signal.

3. What if the response is lower than expected (lower than expected S/B)?

- Make sure that the ligand is prepared correctly, and ensure it is completely dissolved before use.
- Make sure DMSO and other solvent concentrations are not too high (not more than 1% final concentration).
- When testing ligands at high concentrations, make sure the vehicle is compatible.
- Make sure ligand is incubated for the specified time and at the specific temperature.
- Make sure plates are protected from light during incubation.

4. What if the EC_{50} does not match reported values?

- Make sure ligands are incubated at the temperature indicated in the cell line-specific datasheet.
- The source of a ligand, particularly for biologics, can have a large effect on the observed pharmacology.
- Use fresh pipette tips during serial dilutions to avoid carryover.

5. What if the variability between replicates is high?

- Make sure dispensing equipment is properly calibrated and proper pipetting technique is used.

- Make sure to avoid cell clumps when plating. Uneven number of cells in the wells can cause high variability.

6. Do you perform any quantitative expression analysis of your recombinant cell lines?

- No. Expression level is crucial to induce the correct response to its ligands. We select clones based on agonist response rather than the expression level.

7. Can these assays be run in 96-, 384-, and 1536-well formats?

- Yes. These assays can be used in high-throughput format, but correct automation is required for each format. Guidelines on suggested parameters are provided but may need to be optimized for each instrument.

For questions on using this product, please contact Technical Support at 1.866.448.4864 or DRX_SupportUS@eurofinsUS.com

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Contact Information

Eurofins DiscoverX

42501 Albrae Street, Fremont, CA 94538

Web: discoverx.com/gpcr

Phone: 1.866.448.4864

Technical Support: DRX_SupportUS@eurofinsUS.com

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