# CellSensor™ CRE-*bla* CHO-K1 Cell–Based Assay

Catalog Number K1129

Doc. Part No. K1129.pps Pub. No. MAN0003079 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

CellSensor™ CRE-*bla* CHO-K1 cells contain a beta-lactamase reporter gene under control of a CRE response element that is stably integrated into CHO-K1 cells. CRE-*bla* CHO-K1 cells have been functionally validated for Z′-Factor and $EC_{50}$ concentrations of Forskolin.

## Overview of GeneBLAzer™ GPCR cell-based assays

The GeneBLAzer™ GPCR cell-based assay provides a highly accurate, sensitive, and easy-to-use method of monitoring cellular response to drug candidates or other stimuli (Zlokarnik, 1998). The core of the GeneBLAzer™ assay technology is a beta-lactamase (bla) fluorescence resonance energy transfer (FRET) substrate that generates a ratiometric reporter response with minimal experimental noise. In addition to the dual-color (blue/green) readout of stimulated and unstimulated cells, this ratiometric method reduces the absolute and relative errors that can mask the underlying biological response of interest. Such errors include variations in cell number, transfection efficiency, substrate concentration, excitation path length, fluorescence detectors, and volume changes. The GeneBLAzer™ GPCR assay technology has been proven in high-throughput screening (HTS) campaigns for a range of target classes, including G-protein coupled receptors (GPCRs) (Kunapuli, 2003; Xing, 2000), nuclear receptors (Qureshi, 2003; Peekhaus, 2003; Chin, 2003), and kinase signaling pathways (Whitney, 1998).

## Contents and storage

**Table 1 CellSensor™ CRE-*bla* CHO-K1 cells (Cat. No. K1129)**

| Contents | Amount | Storage |
|---|---|---|
| CellSensor™ CRE-*bla* CHO-K1 cells (Cat. No. K1129) | 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** | Blasticidin 5 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.

| 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** | |
| LiveBLAzer™-FRET B/G Loading Kit:<br>• LiveBLAzer™-FRET B/G Substrate (CCF4-AM) (Cat. No. K1089)[1]<br>• DMSO for Solution A<br>• Solution B<br>• Solution C | K1095[2] |
| Solution D | K1156 |
| Recovery™ Cell Culture Freezing Medium | 12648010 |
| DMEM, high glucose, GlutaMAX™ Supplement, pyruvate | 10569010 |
| Opti-MEM™ I Reduced Serum Medium | 31985088 |
| DMSO | Fluka 41647 |
| Fetal bovine serum (FBS), dialyzed, tissue-culture grade **(Do Not Substitute.)** | 26400036 |
| MEM Non-Essential Amino Acids Solution (100X) | 11140050 |
| HEPES (1 M, pH 7.3) | 15630080 |
| 0.05% Trypsin-EDTA | 25300054 |
| Forskolin | Sigma F6886 |
| Blasticidin (antibiotic) | R21001 |

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

## Cell culture conditions

### 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 | Assay Medium | Growth Medium | Thawing Medium | Freezing Medium |
|---|---|---|---|---|
| DMEM, high glucose, GlutaMAX™ Supplement, pyruvate | 90% | 90% | 90% | — |
| Dialyzed FBS (Do not substitute.) | 10% | 10% | 10% | — |
| NEAA | 0.1 mM | 0.1 mM | 0.1 mM | — |
| Hepes (pH 7.3) | 25 mM | 25 mM | 25 mM | — |
| Penicillin (antibiotic) | 100 U/mL | 100 U/mL | 100 U/mL | — |
| Streptomycin (antibiotic) | 100 µg/mL | 100 µg/mL | 100 µg/mL | — |
| Recovery™ Cell Culture Freezing Medium | — | — | — | 100% |
| Blasticidin | — | 5 µg/mL | — | — |

### 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 5% 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 3). 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 Forskolin 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 2 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 16–20 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 Reviewer: in Assay Medium with 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 Mixture | 1. Combine 6 µL of 1 mM LiveBLAzer™-FRET B/G (CCF4-AM) (Solution A) + 60 µL of Solution B, then vortex.<br>2. Add 904 µL of Solution C, then vortex.<br>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 7. | | | |
| **Step 8:** Analyze data | See "Data analysis" on page 7. | | | |

**Table 3 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 16–20 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 5 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.<br>2. Add 904 µL of Solution C, then vortex.<br>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 7. | | | | |
| **Step 9:** Analyze data | See “Data analysis” on page 7. | | | | |

## Detailed assay protocol

### Plate cells

1. 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 16–20 hours.
4. Proceed to the appropriate assay procedure.
   - For the Agonist assay—see “Agonist assay plate setup” on page 5.
   - For the Antagonist assay—see “Antagonist assay plate setup” on page 6.

### 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 5 hours. Then proceed to "Substrate preparation, loading, and incubation" on page 6.

### 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 5 hours. Then proceed to "Substrate preparation, loading, and incubation" on page 6.

## 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.

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**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.

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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**.

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