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1.0 MATERIALS SUPPLIED

Description:	GeneBLAzer® CMV-*bla* CHO-K1 cell line has been engineered to express the beta-lactamase (*bla*) reporter gene under the control of the CMV promoter. A stable population was obtained using 1 mg/ml Geneticin® (G418) selection followed by isolation of a clone by fluorescence-activated cell sorting (FACS). This cell line constitutively expresses beta-lactamase.
Product Number:	K1100
Shipping Condition:	Dry Ice
Storage Condition:	Liquid Nitrogen. **Immediately upon receipt, cells must be stored in liquid nitrogen. Cells cannot be stored at -80°C as they will quickly lose viability.**
Quantity:	~3,000,000 cells (3×10^6 cells/ml)
Cell Line Name:	CMV-*bla* CHO-K1
Function:	Used as a positive control for beta-lactamase expression
Growth Properties:	Adherent
Cell Phenotype:	Epithelial
Antibiotic Selection:	Geneticin® (G418)
***Mycoplasma* Testing:**	Negative
Biosafety Level:	1

2.0 MATERIALS REQUIRED, BUT NOT SUPPLIED

Media/Reagents	Recommended Source	Cat. no.
LiveBLAzer™ FRET B/G Loading Kit containing: LiveBLAzer™ FRET B/G Substrate (CCF4-AM) (5 mg) DMSO for Solution A Solution B Solution C	Invitrogen	K1030 Other sizes and Loading Kits containing CCF2-AM substrate are available
Cell Culture Freezing Medium	Invitrogen	11101-011
DMEM (high-glucose)	Invitrogen	11965-092
DMSO	Fluka	41647
Fetal Bovine Serum (FBS), dialyzed, tissue culture grade **(DO NOT SUBSTITUTE)**	Invitrogen	26400-044
Geneticin® (G418)	Invitrogen	10131-027
HEPES (1 M, pH 7.3)	Invitrogen	15630-080
Non-essential Amino Acids (NEAA)	Invitrogen	11140-050
Penicillin/Streptomycin	Invitrogen	15140-122
Phosphate-buffered saline without calcium and magnesium [PBS(-)]	Invitrogen	14190-136
Solution D	Invitrogen	K1156 or K1157
Sodium Pyruvate	Invitrogen	11360-070
0.5% Trypsin/EDTA	Invitrogen	25300-054
Wild type CHO-K1 Cells	ATCC	CCL-61

Consumables	Recommended Source	Cat. no.
Black-wall, clear-bottom, 96-well assay plates (with low fluorescence background)	Costar	3603
Compressed Air	Various	—
Conical tubes, 15 ml, sterile	Various	—
1.8-ml Internally threaded cryogenic vials	Various	—
Tissue culture flasks	Various	—

Equipment	Recommended Source
Class II Biological Safety Cabinet	Various
Dual wavelength bottom-reading fluorescence plate reader	Various
Filters (see **Section 5.3**)	Chroma Technologies
Hemacytometer (or another cell counting method)	Various
Humidified 37°C/5% CO_2 Incubator	Various
Inverted Microscope	Various
Liquid Nitrogen Tank	Various

2.1 Optional Equipment and Materials

- Epifluorescence or fluorescence-equipped microscope

3.0 CELL CULTURE CONDITIONS

3.1 Media Required

	Growth Medium (+)	Growth Medium (-)/Assay Medium
DMEM, high glucose	90%	90%
Dialyzed FBS	10%	10%
NEAA	0.1 mM	0.1 mM
Sodium Pyruvate	1 mM	1 mM
HEPES (pH 7.3)	25 mM	25 mM
Penicillin	100 U/ml	100 U/ml
Streptomycin	100 µg/ml	100 µg/ml
Geneticin®	500 µg/ml	--

3.2 Methods Used

Please follow these methods exactly, as they have been validated specifically for optimal performance of this cell line. At first opportunity, create and store an aliquot of cells for back-up.

3.2.1 Thawing Method

1. Place 14 ml of Growth Medium (-) into a T75 flask.
2. Place the flask in a 37°C/5% CO_2 incubator for 15 minutes to allow medium to equilibrate to the proper pH and temperature.
3. Remove vial of cells to be thawed from liquid nitrogen and rapidly thaw by placing in a 37°C 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 dropwise into 10 ml of Growth Medium (-) in a sterile 15 ml conical tube.
6. Centrifuge the cells at 200 x *g* for 5 minutes.
7. Aspirate supernatant and resuspend the cell pellet in 1 ml of fresh Growth Medium (-).
8. Transfer contents to the T75 tissue culture flask containing pre-equilibrated Growth Medium (-) and place flask in the 37°C/5% CO_2 incubator.
9. At first passage, switch to Growth Medium (+).

3.2.2 Propagation Method

1. Cells should be fed or passaged at least twice a week. Cells should be maintained between 10% and 90% confluence. **Do not allow cells to reach confluence.**
2. To passage cells, aspirate medium, rinse once with PBS(-), add Trypsin/EDTA (3 ml for a T75 flask, 5 ml for a T175 flask and 8 ml for T225 flask) and swirl to coat the cells evenly. Cells usually detach after ~3-5 minutes exposure to Trypsin/EDTA. Add an equal volume of growth medium to inactivate trypsin.
3. Verify under a microscope that cells have detached and clumps have completely dispersed. If necessary, gently pipette up and down to disperse clumps.
4. Spin down cells and resuspend in Growth Medium.

3.2.3 Freezing Method

1. Harvest the cells as described in **Section 3.2.2, Step 2**.
2. Count the cells.
3. Spin cells down (200 x *g*, 5 min.) and resuspend in 4°C Cell Culture Freezing Medium at 3 x 10^6 cells/ml.
4. Dispense 1 ml aliquots into cryogenic vials.
5. Place vials in an insulated container (for slow cooling) and store overnight at -80°C.
6. Transfer to liquid nitrogen the next day for storage.

4.0 ASSAY PROCEDURE

The following instructions outline the recommended procedure for determining beta-lactamase activity in a 96-well Plate. We recommend at least 5 wells per experimental condition.

4.1 Cells to Wells (Day 1)

1. Harvest cells from culture in Growth Medium.
2. Resuspend the cells in Assay Medium at a density of 5 x 10^5 cells/ml.
3. Add 100 µl (50,000 cells) of cell suspension per well into a 96-well tissue culture treated black-wall, clear-bottom plate.
4. In a separate set of wells on the same plate, add 100 µl (50,000 cells) per well of wild-type CHO-K1 cell suspension in Assay Medium.
5. In a separate set of wells on the same plate, add 100 µl/well of Assay Medium.
6. Incubate cells in a humidified incubator at 37°C/5% CO_2 overnight.

4.2 Substrate Loading (Day 2)

Preparation of 6X LiveBLAzer™ FRET B/G (CCF4-AM) or CCF2-AM Loading Solution and cell loading should be done in the absence of direct strong lighting. Turn off the light in the hood.

4.2.1 LiveBLAzer™ FRET B/G (CCF4-AM) or CCF2-AM Loading Protocol

This protocol is designed for loading cells with LiveBLAzer™ FRET B/G (CCF4-AM), using the following solutions. Please see **Section 5.1** for more details. This protocol is written for LiveBLAzer™ FRET B/G (CCF4-AM), however, CCF2-AM may be subsitituted.

Reagents	Storage and Handling
LiveBLAzer™ FRET B/G Substrate	The product is supplied as a dried powder. The molecular weight of LiveBLAzer™ FRET B/G (CCF4-AM) is 1096 g/mole; the molecular weight of CCF2-AM is 1082 g/mole Store the product at -20°C, desiccated and protected from light.
Dry DMSO	Used to dissolve LiveBLAzer™ FRET B/G (CCF4-AM)for preparation of Solution A.
Solution A	LiveBLAzer™ FRET B/G (CCF4-AM) stock solution; 1 mM LiveBLAzer™ FRET B/G (CCF4-AM) in dry DMSO. Prepare a 1 mM LiveBLAzer™ FRET B/G (CCF4-AM) stock solution in dry DMSO. Store the LiveBLAzer™ FRET B/G (CCF4-AM) stock solution at -20°C, desiccated, protected from light. Before each use, let frozen stock solution warm to room temperature and remove desired amount of reagent. Immediately recap the vial after each use to reduce moisture uptake and return to -20°C storage. Stored under these conditions, Solution A is stable for approximately three months. Once thawed, Solution A may appear slightly yellow. This is normal.
Solution B	Store the reagent at room temperature (18–22°C) protected from direct light. Mix thoroughly before use. Under cold lab conditions [colder than 18°C (65°F)], the solution may freeze or a white precipitate may form. In this case, warm while stirring the solution (~35°C) until thawed and the precipitate dissolves.
Solution C	Keep tightly closed and store in a cool, dark dry place. Use the reagent at room temperature (18–22°C) protected from direct light.
Solution D	Optional: Solution D is a nonspecific anion exchange blocker that can be added to the loading solution to prevent undesired export of the substrate from the cell in cell types with active multidrug transporters. Store stock solution at -20°C.

4.2.2 Preparation of 6X LiveBLAzer™ FRET B/G (CCF4-AM) Loading Solution

1. Add 12 µl of Solution A to 60 µl of Solution B and vortex.
2. Add 898 µl Solution C to the combined solutions from Step 1. Vortex.
3. Add 30 µl of Solution D to the combined solutions from Step 2. Vortex.

4.2.3 Cell Loading

1. Remove assay plate from incubator and allow to equilibrate to room temperature prior to loading.
2. Add 20 µl of the 6X loading solution to each well (2 µM LiveBLAzer™ FRET B/G (CCF4-AM)).

4.3 Incubation

1. Cover the plate to protect it from light and evaporation.
2. Incubate at room temperature for 1-2 hours.

Note: Handle the plate gently and do not touch the bottom.

4.4 Detection

All measurements are made at room temperature from the bottom of the wells, preferably in 96-well black-walled, clear-bottom assay plates with low fluorescence background. Before reading the plate, remove dust from the bottom with compressed air.

Note: Some plates/fluorescence plate readers experience edge effects which may affect data. If edge effects are noticed, plate layout should be considered when setting up the assay.

4.4.1 Instrumentation, Filters and Plates

- Almost all dual wavelength, bottom read fluorescence plate readers can be used to detect beta-lactamase using LiveBLAzer™ FRET B/G (CCF4-AM) or CCF2-AM.
- Note: If you are uncertain of whether your instrument can be used for this assay, please contact Technical Support for assistance. Filters for fluorescence plate reader:

For ratiometric readout using a fluorescence plate reader:

Excitation filter: 409/20 nm
Emission filter: 460/40 nm
Emission filter: 530/30 nm

4.4.2 Reading an Assay Plate

1. Set the fluorescence plate reader to bottom-read mode and for two scans per cycle.
2. Allow the lamp in the fluorescence plate reader to warm up for at least 10 min. before making measurements.
3. Use the following filter selections:

	Scan 1	Scan 2
Purpose:	Measure fluorescence in the blue channel	Measure fluorescence in the green channel
Excitation filter:	409/20 nm	409/20 nm
Emission filter:	460/40 nm (using the gain determined during calibration)	530/30 nm (using the gain determined during calibration)

Typically, set the fluorescence plate reader to 5 reads/well.

5.0 DATA ANALYSIS

5.1 Background Subtraction

Background subtraction for both channels (460 nm and 530 nm) is essential to obtain meaningful data. This can be accomplished either automatically using software connected to the fluorescence plate reader, or manually after each assay plate has been read.

Use the assay plate layout to identify the location of the Cell-free Control wells. These control wells are used for background subtraction.

5.1.1 Automatic background subtraction

Use the assay plate layout to designate appropriate Cell-free Control wells and enable background subtraction for both sets of emission scans.

5.1.2 Manual background subtraction

1. Determine the average emission from the Cell-free Control wells at both 460 nm (Average Em_{460} Background) and 530 nm (Average Em_{530} Background).
2. Calculate the Net CMV-*bla* CHO-K1 Em_{460} and Em_{530} values and the Net CHO-K1 Em_{460} and Em_{530} values, as shown below:

CMV-*bla* CHO-K1 Cells

Net CMV-*bla* CHO-K1 Em_{460} = Em_{460} of CMV-*bla* CHO-K1 sample – Average Em_{460} Background

Net CMV-*bla* CHO-K1 Em_{530} = Em_{530} of CMV-*bla* CHO-K1 sample – Average Em_{530} Background

CHO-K1 Cells

Net CHO-K1 Em_{460} = Em_{460} of CHO-K1 sample – Average Em_{460} Background
Net CHO-K1 Em_{530} = Em_{530} of CHO-K1 sample – Average Em_{530} Background

5.2 Calculating the Response Ratio

1. Calculate the CMV-*bla* CHO-K1 Emission Ratio for each well, as shown below:

$$\textbf{CMV-}\textit{bla}\textbf{ CHO-K1 Emission Ratio} = \frac{\textbf{Net CMV-}\textit{bla}\textbf{ CHO-K1 Em}_{460}}{\textbf{Net CMV-}\textit{bla}\textbf{ CHO-K1 Em}_{530}}$$

2. Calculate the CHO-K1 Emission Ratio for each well, as shown below:

$$\textbf{CHO-K1 Emission Ratio} = \frac{\textbf{Net CHO-K1 Em}_{460}}{\textbf{Net CHO-K1 Em}_{530}}$$

3. Calculate the Mean CHO-K1 Emission Ratio for each assay plate, as shown below:

$$\textbf{Mean CHO-K1 Emission Ratio} = \frac{\textbf{Sum of CHO-K1 Emission Ratios}}{\textbf{Total number of wells containing CHO-K1 cells}}$$

4. Calculate the Response Ratio for each well of interest, as shown below:

$$\textbf{Response Ratio} = \frac{\textbf{CMV-}\textit{bla}\textbf{ CHO-K1 Emission Ratio (Em}_{460}\textbf{/Em}_{530}\textbf{)}}{\textbf{Mean CHO-K1 Emission Ratio (Em}_{460}\textbf{/Em}_{530}\textbf{)}}$$

5.3 Visual Observation of Intracellular LiveBLAzer™ FRET B/G (CCF4-AM)

***Note*:** Microscopic visualization of cells will cause photobleaching. Always read the assay plate in the fluorescence plate reader before performing microscopic visualization.

An inverted microscope equipped for epifluorescence and either a xenon or mercury excitation lamp is typically required to view the LiveBLAzer™ FRET B/G (CCF4-AM) signal in cells. To visually inspect the cells, a long-pass filter passing blue and green fluorescence light is needed 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).

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).

6.0 PURCHASER NOTIFICATION

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Use of Genetically Modified Organisms (GMO)

Information for European Customers The CMV-*bla* CHO-K1 cell line(s) are genetically modified with the plasmid pCMV-blaX-neo. 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.