

## CellSensor® CRE-*bla* FreeStyle™ 293F Cell Line

Cat. no. K1636

This cell-based assay has been thoroughly tested and validated by Invitrogen and is suitable for immediate use in a screening application. The following information illustrates the high level of assay testing completed and the validation of assay performance under optimized conditions.

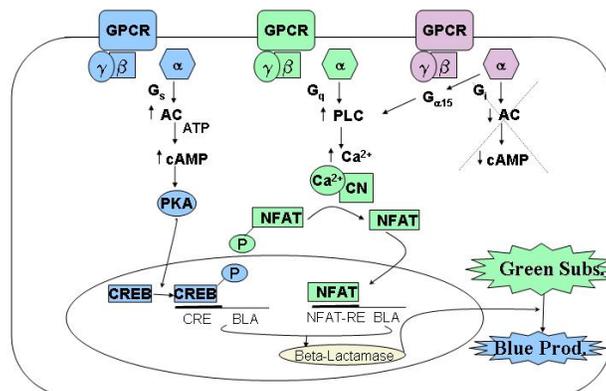
### Pathway Description

The G protein-coupled receptor (GPCR) superfamily contains over 1,000 members and is the largest known class of molecular targets with proven therapeutic value. G proteins are trimeric, containing  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits. G proteins can be categorized into several classes based on their  $\alpha$  subunit. Three of the best characterized classes are  $G_q$ ,  $G_s$ , and  $G_i$ , for their ability to stimulate phospholipase, stimulate adenylyl cyclase, or inhibit adenylyl cyclase, respectively. When adenylyl cyclase is stimulated, cAMP concentrations rise. In the CRE-*bla* Freestyle 293F cell line, the beta-lactamase gene is linked to a cAMP response element (CRE). As a result, when intracellular cAMP levels rise, beta-lactamase will be produced.

The CRE-*bla* Freestyle 293F line can be used as a building block to create specific Gs coupled GPCR assays when the GPCR of interest is transfected into these cells.

### Cell Line Description

The CellSensor® CRE-*bla* FreeStyle™ 293T cell line contains a beta-lactamase reporter gene under control of the CRE response element stably integrated into FreeStyle™ 293T cells. This cell line is a clonal population isolated in response to forskolin by flow cytometry. This cell line has also been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, substrate loading time, and validated for Z' and EC<sub>50</sub> concentrations of forskolin.



## Validation Summary

Testing and validation of this assay was evaluated using LiveBLazer™-FRET B/G Substrate.

### 1. Primary agonist dose response under optimized conditions

Forskolin EC<sub>50</sub> = 872 nM  
Z'-Factor (EC<sub>100</sub>) = 0.92  
Response Ratio = 32.46

Optimum cell no. = 20K cells/well  
Optimum [DMSO] = 0.5%  
Optimum Stim. Time = 5 hours  
Max. [Stimulation] = 13.33 uM

### 2. Cell culture and maintenance

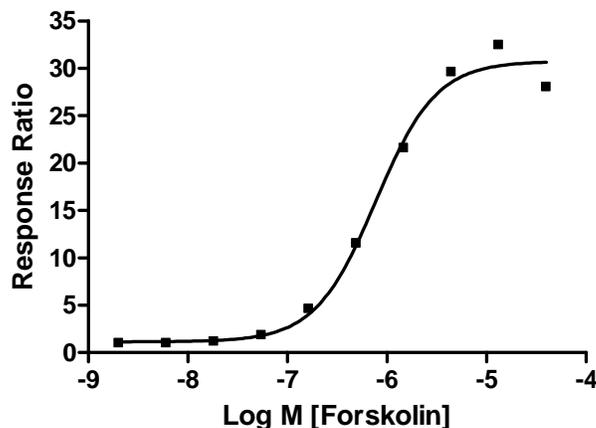
See *Cell Culture and Maintenance Section and Table 1*

## Assay Testing Summary

3. Assay performance with variable cell number
4. Assay performance with variable stimulation time
5. Assay performance with variable substrate loading time
6. Assay performance with variable DMSO concentration

## Primary Agonist Dose Response

Figure 1 –CRE-*bla* FreeStyle™ 293F dose response to forskolin under optimized conditions



CRE-*bla* FreeStyle™ 293F cells (20,000 cells/well) were plated in a 384-well plate and stimulated with forskolin over the indicated concentration range in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLazer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted for the indicated concentrations of forskolin (data is average of data collected on three separate days).

## Cell Culture and Maintenance

Thaw cells in Growth Medium without Blasticidin and culture them in Growth Medium with Blasticidin. Pass or feed cells at least twice a week and maintain them in a 37°C/5% CO<sub>2</sub> incubator. Maintain cells between 10% and 90% confluency. Do not allow cells to reach confluence

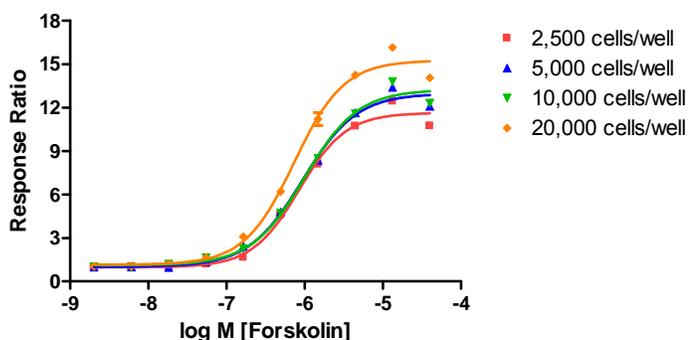
*Note:* We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. Freeze cells at 2 x 10<sup>6</sup> cells/ml in Freezing Medium. For optimal cell line performance, use Dialyzed FBS (Invitrogen # 26400-036). For detailed growth and maintenance directions, please refer to the protocol.

**Table 1 – Cell Culture and Maintenance**

Component	Growth Medium	Assay Medium	Freezing Medium	1X Matrigel
DMEM	90%	90%	—	99.75%
Dialyzed FBS Do not substitute!	10%	10%	—	—
NEAA	0.1 mM	0.1 mM	—	—
HEPES (pH 7.3)	25 mM	25 mM	—	—
Penicillin (antibiotic)	100 U/ml	100 U/ml	—	—
Streptomycin (antibiotic)	100 µg/ml	100 µg/ml	—	—
Blasticidin (antibiotic)	5 µg/ml (do not thaw with Blasticidin)	—	—	—
Cell Culture Freezing Medium	—	—	100%	—
Matrigel™ Matrix	—	—	—	0.25%

**Assay Performance with Variable Cell Number**

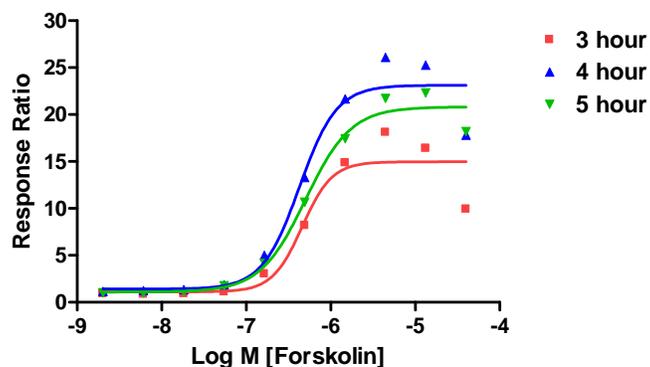
**Figure 2 - CRE-*bla* FreeStyle™ 293F response to forskolin using 2,500, 5,000, 10,000, and 20,000 cells/well**



CRE-*bla* FreeStyle™ 293F cells were plated at 2,500, 5,000, 10,000, and 20,000 cells/well in a 384-well format. Cells were then stimulated with forskolin at various concentrations in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each cell number against the various concentrations of forskolin.

**Assay Performance with Variable Stimulation Time**

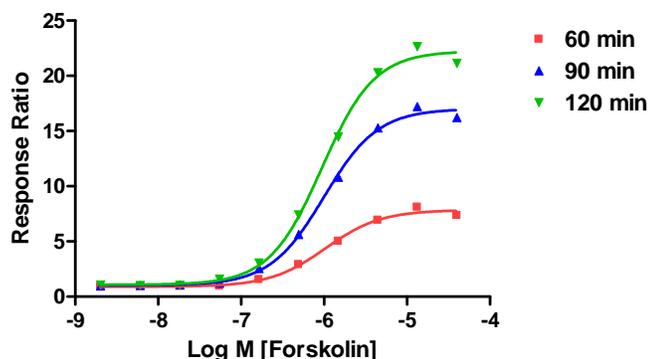
**Figure 3 – CRE-*bla* FreeStyle™ 293F dose response to Forskolin using 3, 4 and 5 hour stimulation times**



CRE-*bla* FreeStyle™ 293F cells (20,000 cells/well) were plated in a 384-well assay plate. Plates were stimulated for 3, 4 or 5 hrs with forskolin in 0.5% DMSO and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each stimulation time.

## Assay Performance with Variable Substrate Loading Time

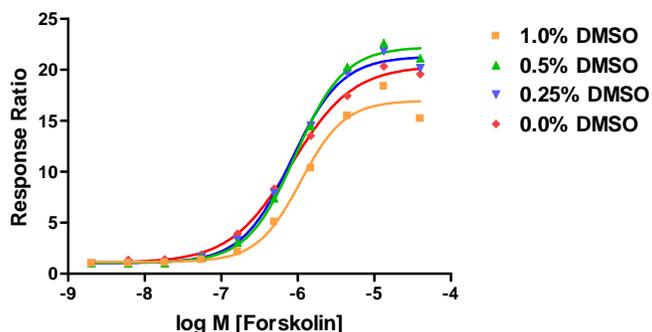
Figure 4 — CRE-*bla* FreeStyle™ 293F response to forskolin with 1, 1.5, and 2 hour substrate loading times



CRE-*bla* FreeStyle™ cells were plated at 20,000 cells/well in a 384-well format. Cells were stimulated with forskolin at various concentrations in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1, 1.5 or 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various substrate loading times were obtained using a standard fluorescence plate reader and the Response Ratios plotted for the indicated substrate loading times.

## Assay Performance with Variable DMSO Concentration

Figure 5 – CRE-*bla* FreeStyle™ 293F response to forskolin using 0, 0.25, 0.5 and 1% DMSO



CRE-*bla* FreeStyle™ 293F cells (20,000 cells/well) were plated in a 384-well plate and treated with the indicated concentrations of forskolin with final DMSO concentrations ranging from 0% to 1%. Plates were stimulated for 4 hrs and loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each forskolin concentration for each DMSO concentration.