

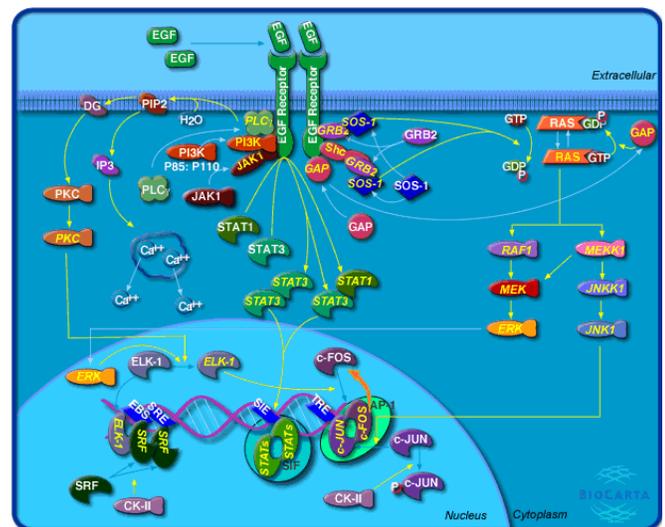
CellSensor® *c-fos-bla* ME180 Cell Line

Cat. no. K1650

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

c-fos is an immediate-early response gene, induced well before cells enter the S phase and replicate their DNA. The gene encodes the transcription factor c-Fos, which induces expression of many genes encoding proteins necessary for cells to progress through the cell cycle. The expression of immediate early response genes is affected by many extracellular stimuli, such as serum, growth factors, cytokines, and UV radiation. These cells are responsive to several ligands, and the validation was done using epidermal growth factor (EGF). Signaling takes place through an EGF Receptor complex.



Cell Line Description

The CellSensor® *c-fos-bla* ME180 cell line contains a beta-lactamase reporter gene under control of the *c-fos* promoter stably integrated into ME180 cells. This cell line is a clonal population isolated in response to EGF 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 EC₅₀ concentrations of EGF. Additional testing information with a panel of ligands is also provided.

Validation Summary

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

1. Primary agonist dose response under optimized conditions

EGF EC ₅₀	= 0.41 ng/mL
Z'-Factor (EC ₁₀₀)	= 0.85
Response Ratio	= 9.07
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= 0.5%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 150 ng/mL

2. Cell culture and maintenance

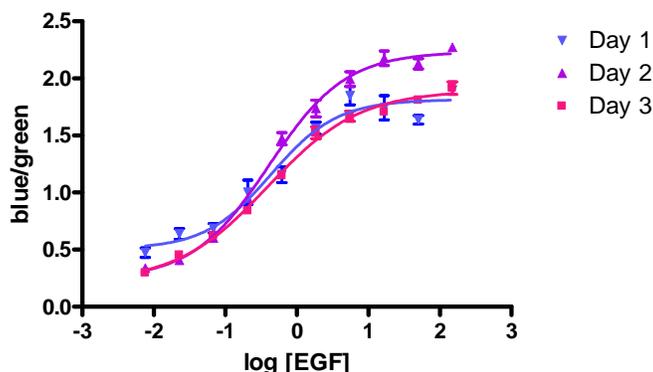
See *Cell Culture and Maintenance Section and Table 1*

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
7. Ligand Panel

Primary Agonist Dose Response

Figure 1 –c-fos-bla ME180 dose response to EGF under optimized conditions



c-fos-bla ME180 cells (20,000 cells/well) were assayed on three separate days, represented by the three curves shown on the graph. Cells were plated in a 384-well plate and stimulated with EGF over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2.5 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 EGF (n=16 for each data point).

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% CO₂ incubator. Maintain cells between 10% and 90% confluency. Do not allow cells to reach confluence

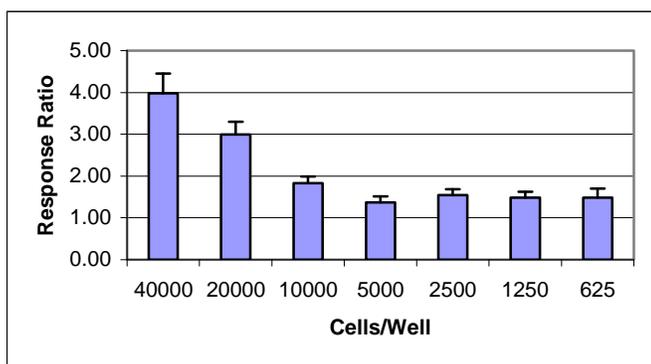
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. Freeze cells at 2 x 10⁶ cells/ml in Freezing Medium. For optimal cell line performance, use Dialyzed FBS (Invitrogen # 26400-036). For detailed growth and maintenance directions, please refer to the protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	Freezing Medium
DMEM	90%	—	—
Opti-MEM® 1	—	99.5%	—
Dialyzed FBS Do not substitute!	10%	0.5%	—
NEAA	0.1 mM	0.1 mM	—
Sodium pyruvate	1 mM	1 mM	—
HEPES (pH 7.3)	25 mM	10 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%

Assay Performance with Variable Cell Number

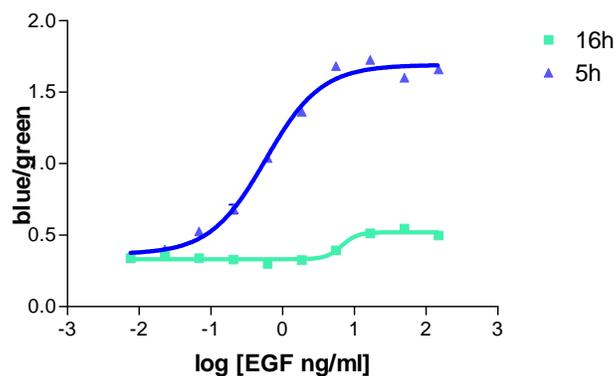
Figure 2 — *c-fos-bla* ME180 response to EGF using 625, 1,250, 2,500, 5,000, 10,000, 20,000 and 40,000 cells/well



c-fos-bla ME180 cells were plated at 625, 1,250, 2,500, 5,000, 10,000, 20,000 and 40,000 cells/well in a 384-well format. Cells were then stimulated with EGF at 50 ng/ml in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAZer™-FRET B/G Substrate for 2.5 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 fixed concentration of EGF.

Assay Performance with Variable Stimulation Time

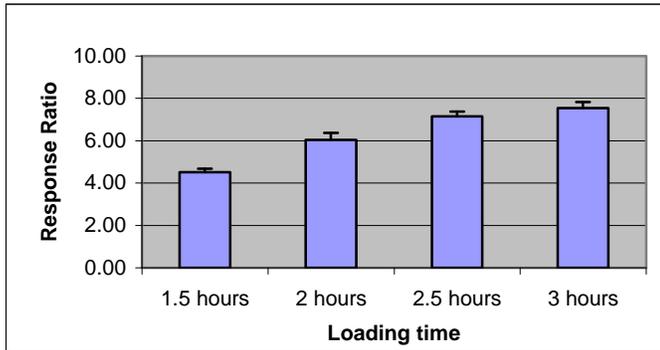
Figure 3 – *c-fos-bla* ME180 dose response to EGF using 5 and 16 hour stimulation times



c-fos-bla ME180 cells (20,000 cells/well) were plated the in a 384-well assay plate. Plates were stimulated for 5 or 16 hrs with EGF in 0.5% DMSO and then loaded for 2.5 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

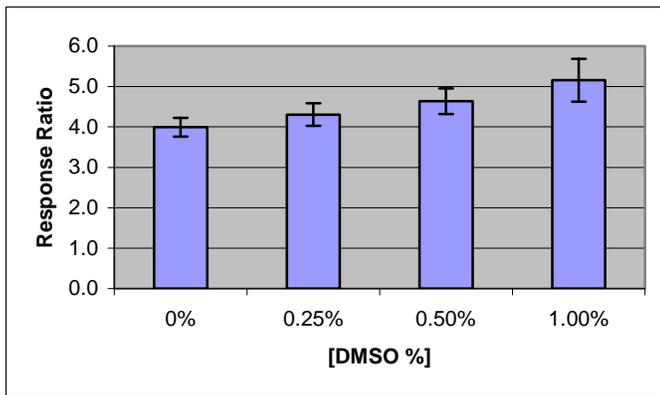
Figure 4 — *c-fos-bla* ME180 response to EGF with 1.5, 2, 2.5 and 3 hour substrate loading times



c-fos-bla ME180 cells were plated at 20,000 cells/well in a 384-well format. Cells were stimulated with 50 ng/ml EGF in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1.5, 2, 2.5 or 3 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

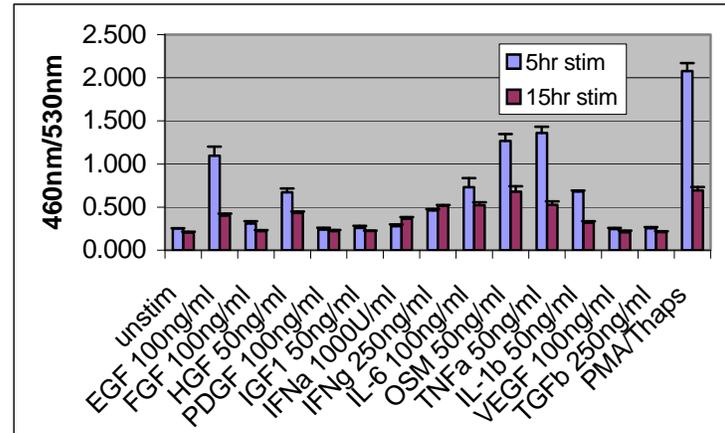
Figure 5 — *c-fos-bla* ME-180 response to EGF using 0, 0.25, 0.5 and 1% DMSO



c-fos-bla ME180 cells (20,000 cells/well) were plated in a 384-well plate and treated with the indicated concentrations of EGF with final DMSO concentrations ranging from 0% to 1%. Plates were stimulated for 5 hrs and loaded for 2.5 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 DMSO concentration.

Ligand Panel Results

Figure 6 — *c-fos-bla* ME180 response to various ligands with 5 and 16 hour stimulation times



c-fos-bla ME180 cells were plated at 20,000 cells/well in a 384-well format. Cells were stimulated with various ligands in the presence of 0.5% DMSO for 5 or 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2.5 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 each ligand tested.