

Validation & Assay Performance Summary



CellSensor[®] *c-fos-bla* HEK293T Cell Line

Cat. no. K1659

CellSensor[®] Cell-Based Assay Validation Packet

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, critical in cell proliferation. The *c-fos* promoter region contains three response elements: a sis-inducible element (SIE), a serum response element (SRE), and a cAMP response element (CRE).

Cell Line Description

The *c-fos-bla* HEK293T cell line stably expresses beta lactamase (*bla*) under the regulation of the *c-fos* promoter. These cells have been shown to be responsive to stimulation with Phorbol 12-myristate 13-acetate (PMA), and will express beta-lactamase upon stimulation by PMA. This cell line has also been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and validated for Z' and EC₅₀ concentrations of IL-1.

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLazer™-FRET B/G Substrate.

1. Primary agonist dose response under optimized conditions (n=3)

PMA EC₅₀ = 1.82 nM
Z'-Factor (EC₁₀₀) = 0.69
Response Ratio = 3.73

Optimum cell no. = 5,000 cells/well
Optimum [DMSO] = 0.0-1.0%
Optimum Stim. Time = 24 hrs
Max. [Stimulation] = 35 nM PMA

2. Cell culture and maintenance

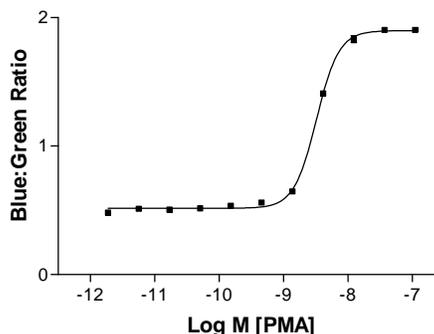
See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

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

Primary Agonist Dose Response

Figure 1 — *c-fos-bla* HEK293T dose response to PMA under optimized conditions



c-fos-bla HEK293T cells (5,000 cells/well) were plated in a 384-well format and were stimulated with PMA over the indicated concentration range in the presence of 0.5% DMSO for 6 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 460nm/530nm ratio plotted for the indicated concentrations of PMA. EC₅₀ was determined to be 0.55pM.

Cell Culture and Maintenance

Thaw cells in Growth Medium without Geneticin® and culture them in Growth Medium with Geneticin®. 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% confluence.

The *c-Fos* promoter contains a serum response element. Therefore, the cells must be incubated in a low serum environment (OPTI-MEM + 0.5% FBS) for 24 hours prior to the assay to allow the background levels of serum induced beta-lactamase to subside. If this step is omitted, the unstimulated cells will be very blue.

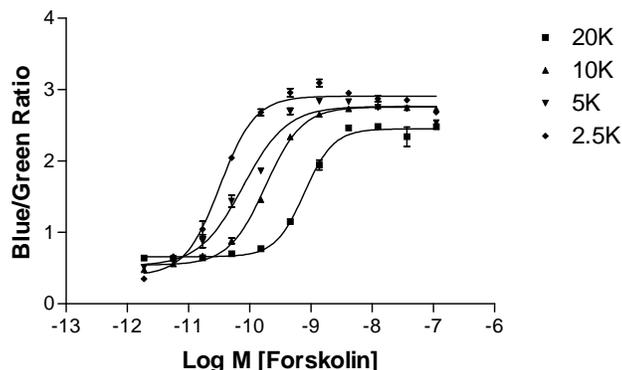
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For more detailed cell growth and maintenance directions, please refer to the customer protocol.

Table 1 – Cell Culture and Maintenance

| Component | Growth Medium | Assay Medium | Freezing Medium |
|--|---------------|--------------|-----------------|
| DMEM | 90% | -- | -- |
| Opti-MEM1 | -- | 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 | 25 mM | -- |
| Penicillin | 100 U/ml | 100 U/ml | -- |
| Streptomycin | 100 µg/ml | 100 µg/ml | -- |
| Blasticidin (antibiotic) | 5 µg/ml | -- | -- |
| Recovery™ Cell Culture Freezing Medium | -- | -- | 100% |

Assay Performance with Variable Cell Number

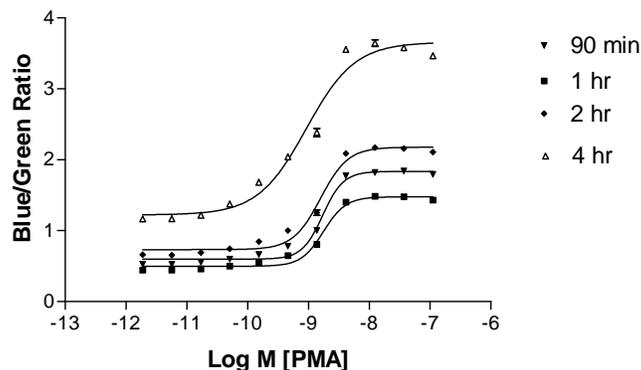
Figure 4 – c-fos-bla HEK293T response to Forskolin with different plating cell numbers/well



c-fos-bla HEK293T cells were plated with indicated number of cells/well in a 384-well format in assay medium. They were stimulated with indicated concentrations of forskolin for 24 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. 5,000 cells/well was found to be optimal.

Assay Performance with Variable Loading Time

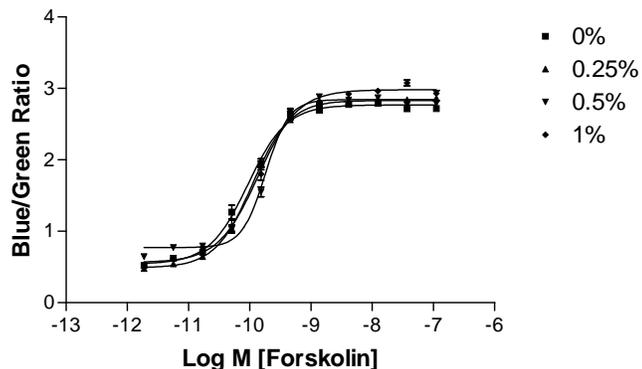
Figure 5 – c-fos-bla HEK293T response to PMA with different loading times



c-fos-bla HEK293T cells were plated at 5,000 cells/well in a 384-well format in assay medium. They were stimulated with the indicated concentration of PMA for 24 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for the indicated time. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader.

Assay Performance with Variable DMSO Concentration

Figure 7 – *c-fos-bla* HEK293T response to PMA with 0, 0.25, 0.50 and 1% DMSO



c-fos-bla HEK293T cells were plated at 5,000 cells/well in a 384-well format in assay medium. They were stimulated with forskolin in the presence of indicated amount of DMSO for 24 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.