Validation & Assay Performance Summary

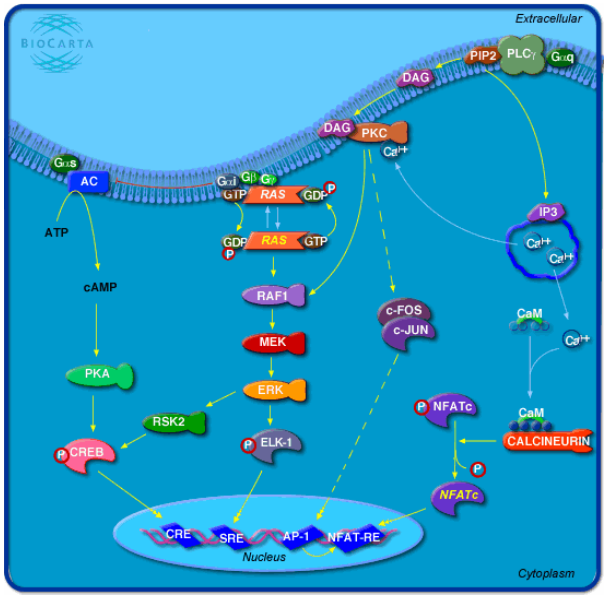
CellSensor®AP1-*bla* HEK 293T Cell Line

Cat. no. K1658

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

Protein Kinase C (PKC) can be up-regulated by various compounds and ligands and has many down stream targets. When Phorbol 12-Myristate 13-Acetate (PMA) is used to stimulate Protein Kinase C, one of the downstream pathways activated is the AP-1 pathway. Stimulation of PKC by PMA leads to dimerization of the c-fos and c-jun proteins to form the AP-1 transcription factor complex which can, in turn, bind to AP-1 response elements to activate transcription of downstream genes.



Cell Line Description

The CellSensor® AP-1-*bla* HEK 293T cell line contains a beta-lactamase reporter gene under control of the Activator Protein-1 (AP-1) response element stably integrated into HEK 293T cells. This cell line has also been tested for assay performance under variable conditions, including DMSO concentration, stimulation time, substrate loading time and validated for Z' and EC_{50} concentrations of PMA.

Validation Summary

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

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

PMA EC_{50}	= 2.87 nM
Z'-Factor (EC_{100})	= 0.80
Response Ratio	= 6.1
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= 0.5%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 1.62 uM

2. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section

Assay Testing Summary

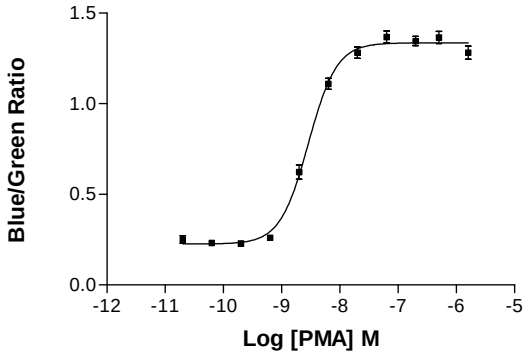
3. **Assay performance with variable PMA stimulation time**

4. **Assay performance with variable substrate loading time**

5. **Assay performance with variable DMSO concentration**

Primary Agonist Dose Response

Figure 1 — AP-1-*bla* HEK 293T dose response to PMA under optimized conditions



AP-1-*bla* HEK 293T cells (20,000 cells/well) were assayed on three separate days. The average of the three days is plotted above. Cells were plated in a 384-well plate and stimulated with PMA 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 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 PMA (n=5 for each data point).

Cell Culture and Maintenance

Thaw and culture cells in Growth Medium. Pass or feed cells at least twice a week and maintain them in a 37°C/5% CO_2 incubator. Maintain cells between 10% and 70% confluency. Do not allow cells to reach confluence. It is not necessary for cells to be maintained in flasks coated in Matrigel, but when the cells are to be assayed, they require Matrigel to remain adhered to the well.

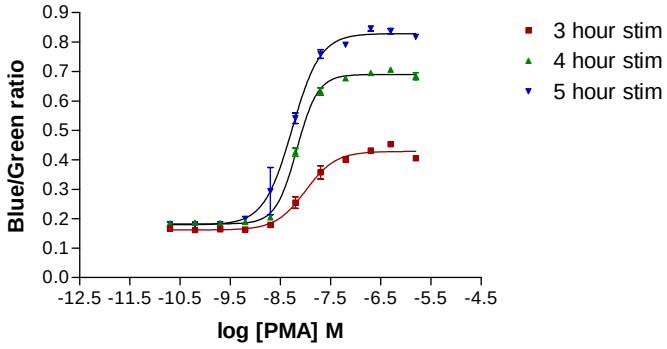
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For optimal cell line performance, use dialyzed FBS (Invitrogen # 26400-010). For detailed growth and maintenance directions, refer to the protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	1X Matrigel
DMEM	90%	90%	99.75%
Dialyzed FBS **Do not Substitute!**	10%	10%	—
NEAA	0.1 mM	0.1 mM	—
Sodium pyruvate	1 mM	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	5 µg/ml	—
Matrigel	—	—	0.25%

Assay Performance with Variable Stimulation Time

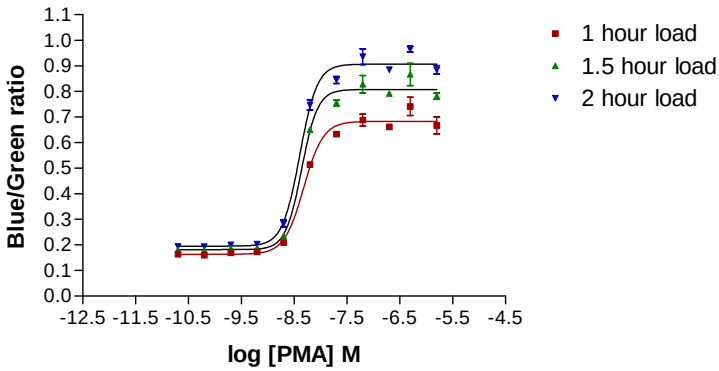
Figure 2— AP-1-*bla* HEK 293T dose response to PMA using 3, 4, and 5 hour stimulation times.



AP-1-*bla* HEK 293T cells (20,000 cells/well) were plated in a 384-well assay plate. Cells were then stimulated for either 3, 4, or 5 hrs with PMA 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 at the indicated concentrations of PMA (n=8 for each data point).

Assay Performance with Variable Substrate Loading Time

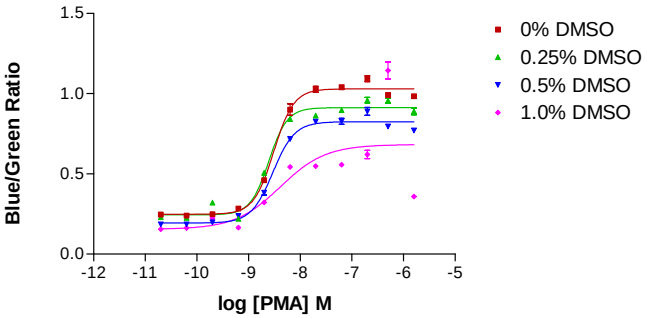
Figure 3 — AP-1-*bla* HEK 293T dose response to PMA with 1, 1.5, and 2 hour substrate loading times



AP-1-*bla* HEK 293T cells were plated at 20,000 cells/well in a 384-well format. Cells were then stimulated with the indicated concentrations of PMA in the presence of 0.5% DMSO for 5 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 each substrate loading time at the indicated concentrations of PMA (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 4 —AP-1-*bla* HEK 293T dose response to PMA using 0, 0.25, 0.5 and 1% DMSO



AP-1-*bla* HEK 293T cells (20,000 cells/well) were plated in a 384-well plate and stimulated with the indicated concentrations of PMA using final DMSO concentrations ranging from 0% to 1%. Plates were stimulated for 5 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 DMSO concentration at the indicated concentrations of PMA (n=8 for each data point).