

Validation & Assay Performance Summary



CellSensor® C/EBP-*bla* FreeStyle™ 293T Cell Line

Cat. no. K1634

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

CCAAT/enhancer-binding protein α (C/EBP α), a basic leucine zipper transcription factor, is involved in the regulation of mitotic growth arrest and differentiation. Mutations in C/EBP α are thought to result in diseases such as human acute myeloid leukemia. C/EBP α is expressed in liver, fat, lung, peripheral leukocytes, epidermis, intestine, and skeletal muscle. C/EBP α also plays a role in energy homeostasis, whereas dominant negative mutations cause altered hepatic glucose and glycogen metabolism as well as defects in white adipose tissue differentiation.

Cell Line Description

The CellSensor® C/EBP-*bla* FreeStyle™ 283T cell line contains a beta-lactamase reporter gene under control of the C/EBP response element stably integrated into FreeStyle™ 293T cells. This cell line validated for EC₅₀ and Z'-Factor under optimized conditions using PMA. This cell line has also been tested under variable experimental conditions, including cell number, stimulation time, and beta-lactamase substrate loading time.

Validation Summary

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

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

PMA EC ₅₀	= 0.75 nM
Z'-Factor (EC ₁₀₀)	= 0.73
Response Ratio	= 4.86
Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= 0.5%
Optimum Stim. Time	= 5 hours
Max. [Inhibition]	= 11.11 nM

2. Determination of LiCl concentration

See Determination of LiCl Section and Figure 2

3. Cell culture and maintenance

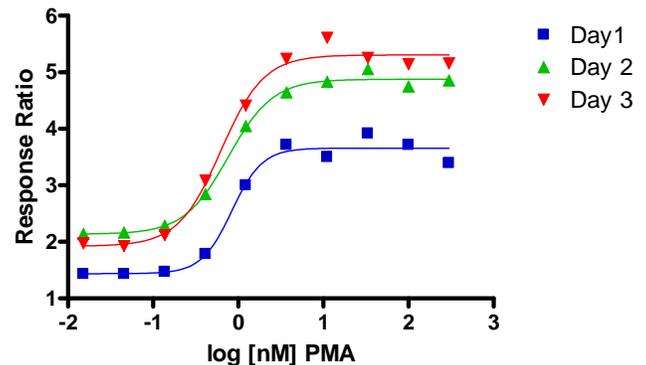
See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

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

Primary Agonist Dose Response

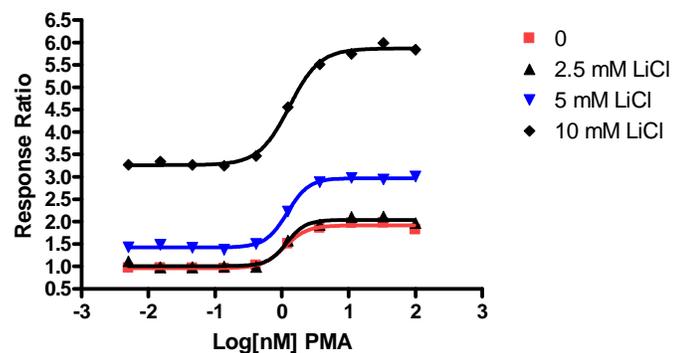
Figure 1 —C/EBP-*bla* FreeStyle™ 293T dose response to PMA under optimized conditions



C/EBP-*bla* FreeStyle™ 293T cells (10,000 cells/well) were assayed on three separate days represented by the three curves shown on the graph. Cells were plated the day of the assay in a 384-well format and stimulated with LiCl (10mM) for 16 hours followed by 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 2.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the 460/530 Emission Ratios plotted for the indicated concentrations of PMA (n=16 for each data point).

Determination of LiCl Concentration

Figure 2 —C/EBP-*bla* FreeStyle™ 293T dose response to PMA with various concentrations of LiCl



C/EBP-*bla* FreeStyle™ 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well format and stimulated with LiCl (0 mM, 2.5 mM, 5 mM or 10 mM) for 16 hours followed by 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 2.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the 460/530 Emission Ratios plotted for the indicated concentrations of PMA (n=16 for each data point).

Cell Culture and Maintenance

Cells are grown in a 90:10 mix of complete growth media: T-STIM™ Culture Supplement with conA (BD Biosciences, 354115). The T-STIM™ Culture Supplement should be kept at 4°C and should not be warmed, due to loss in activity. It should be mixed with growth media just before using with cell cultures. Cell culture medium is changed every other day to replenish fresh IL-2. Cells are

maintained at a cell density between 2×10^4 and 2×10^5 cells/ml.

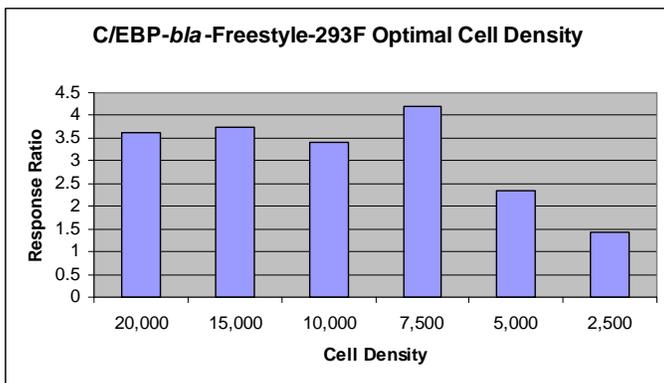
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For proper cell line performance, use dialyzed FBS (Invitrogen# 26400-036). For more detailed cell growth and maintenance directions, please refer to protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	Freezing Medium
DMEM	90%	—	—
Opti-MEM®	—	96.5%	—
Dialyzed FBS Do not substitute!	10%	0.5%	—
NEAA	0.1 mM	0.1 mM	—
Sodium Pyruvate	—	1 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

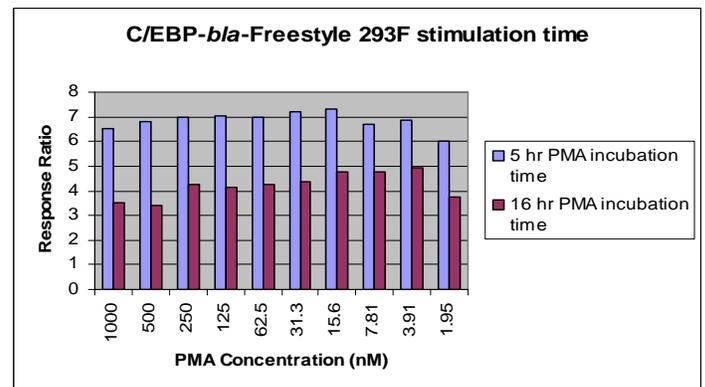
Figure 3 – C/EBP-*bla* FreeStyle™ 293F response to PMA with 2.5K, 5K, 7.5K, 10K, 15K and 20K cells/well



C/EBP-*bla* FreeStyle™ 293F cells were plated the day of the assay at 2,500, 5,000, 7,500, 10,000, 15,000 or 25,000 cells/well in a 384-well format. Cells were then stimulated with LiCl (10mM) for 16 hours followed by PMA (500 nM) 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 460/530. (n=16 for each data point).

Assay Performance with Variable Stimulation Time

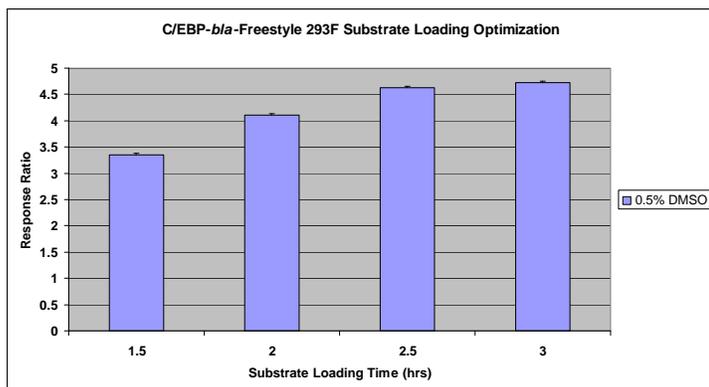
Figure 4 – C/EBP-*bla* FreeStyle™ 293F dose response to PMA with 5 and 16 hour stimulation times



C/EBP-*bla* FreeStyle™ 293F cells (10,000 cells/well) were plated the day of the assay in a 384-well assay plate. Cells were then stimulated with 10 mM LiCl for 16 hours. Plates were treated for 5 or 16 hrs with PMA at the indicated concentrations in the presence of 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. (n=8 for each data point).

Assay Performance with Variable Substrate Loading Time

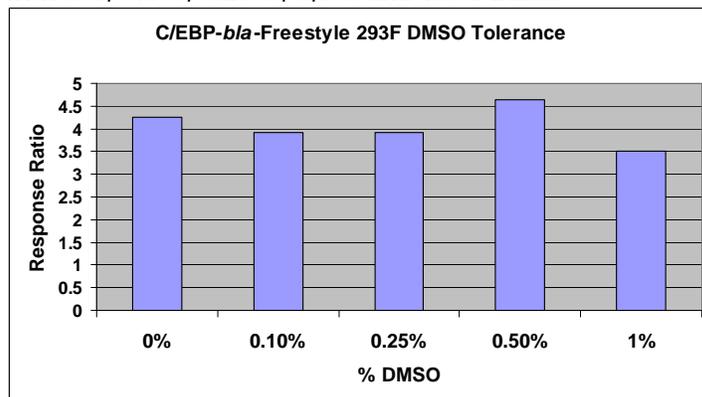
Figure 5 — C/EBP-*bla* FreeStyle™ 293F response to PMA with 1.6, 2, 2.6, and 3 hour substrate loading times



C/EBP-*bla* FreeStyle™ 293F cells were plated the day of the assay at 10,000 cells/well in a 384-well format. Cells were treated with LiCl (10 mM) for 16 hours followed by treatment with PMA (500 nM) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1.6, 2, 2.6, 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 each substrate loading time. (n=4 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 6 — C/EBP-*bla* FreeStyle™ 293F response to PMA with 0%, 0.1%, 0.25%, 0.5% and 1.0% DMSO



C/EBP-*bla* FreeStyle™ 293F cells (10,000 cells/well) were plated the day of the assay in a 384-well assay plate. Cells were treated with LiCl (10 mM) for 16 hours followed by treatment with PMA (500 nM) in the presence of 0%, 0.1%, 0.25%, 0.5% or 1% DMSO for 5 hours. They were 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 were plotted for each DMSO concentration (n=6 for each data point).