Validation & Assay Performance Summary

CellSensor® IL-1-*bla* ECV304 Cell Line

Cat. no. K1645

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

Cell signaling through the IL-1 cytokine signaling pathway is important in immune and inflammatory responses.

Cell Line Description

The CellSensor® IL-1-*bla* ECV304 cell line contains a beta-lactamase reporter gene, which can be stimulated by IL-1, stably integrated into the adherent human urinary bladder cell line ECV304. This cell line was constructed using GenomeScreen™ technology. The GenomeScreen™ system is based on sensitive, single cell detection of gene expression in which a promoterless beta-lactamase reporter gene is randomly integrated into the genome of a cell line. Sequential rounds of sorting are used to isolate cell clones where tagged genes are induced by different agents, in this case IL-1. 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 EC_{50} 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)**

IL-1 EC_{50}	= 0.015 ng/ml
Z'-Factor (EC_{100})	= 0.67
Response Ratio	= 2.27
Optimum cell no.	= 10,000 cells/well
Optimum [DMSO]	= 0.0-1.0%
Optimum Stim. Time	= 6 hrs
Max. [Stimulation]	= 1.25 ng/ml

2. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

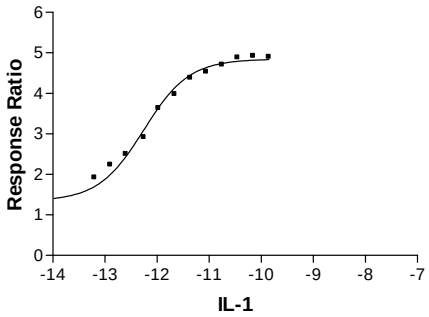
3. **Assay performance with variable cell number**

4. **Assay performance with variable stimulation time**

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

Primary Agonist Dose Response

Figure 1 — IL-1 dose response under optimized conditions



IL-1-*bla* ECV304 cells (10,000 cells/well) were plated in a 384-well format and were stimulated with IL-1 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 IL-1. 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% CO_2 incubator. Maintain cells between 10 and 90% confluence.

All flasks must be coated with 1X Matrigel™ matrix before plating. For 384-well format, it was found the assay performs more robustly without Matrigel™ matrix.

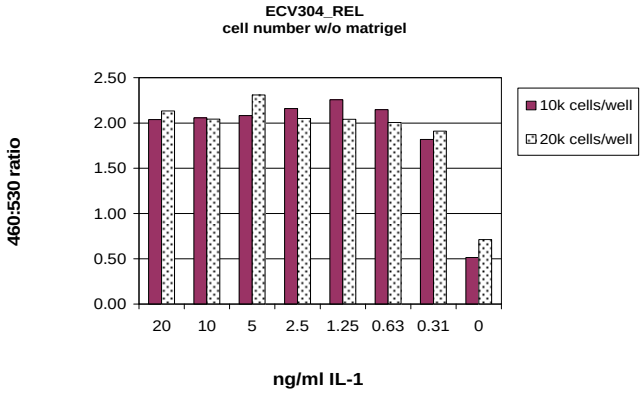
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For more detailed cell growth and maintenance directions, please refer to the customer protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	1X Matrigel™ Matrix
Medium 199	90%	--	--
OptiMEM1, no phenol red	--	99%	99.75%
Dialyzed FBS **(do not substitute!)**	10%	--	--
FBS, Charcoal stripped	--	1%	--
L-glutamine	2 mM	--	--
NEAA	0.1 mM	--	--
Sodium Pyruvate	1 mM	--	--
Penicillin (antibiotic)	100 U/ml	100 U/ml	--
Streptomycin (antibiotic)	100 µg/ml	--	--
Geneticin® (antibiotic)	400 µg/ml		
Matrigel™ Matrix	--	--	0.25%

Assay Performance with Variable Cell Number

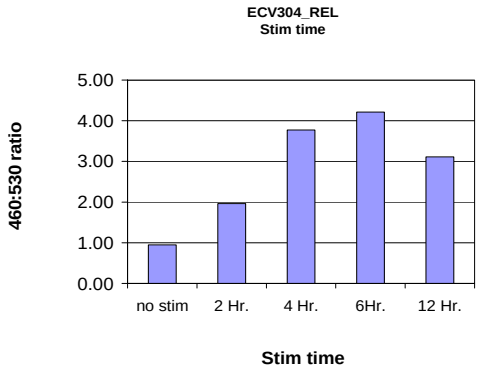
Figure4 — IL-1 response with different plating cell numbers / well



IL-1-*bla* ECV304 cells were plated with indicated number of cells/well in a 384-well format in assay medium. They were stimulated with indicated concentrations of IL-1 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 for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each cell number. 10,000 cells/well was found to be optimal at max stim (1.25ng/mL IL-1).

Assay Performance with Variable Stimulation Time

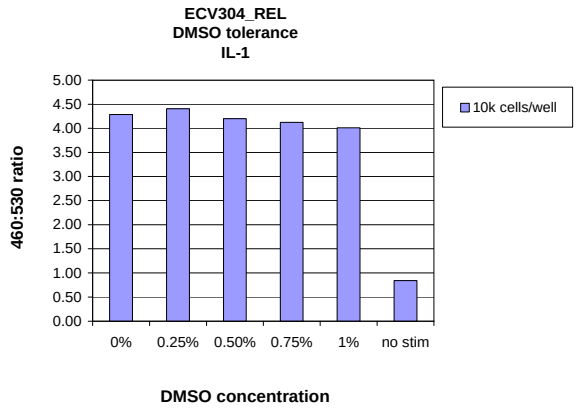
Figure 5 – IL-1 response with different stimulation times



IL-1-*bla* ECV304 cells were plated at 10,000 cells/well in a 384-well format in assay medium. IL-1 concentration was 1.25 ng/mL. 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.

Assay Performance with Variable DMSO Concentration

Figure 7 – IL-1 response with 0, 0.25, 0.5, 0.75 and 1 % DMSO



IL-1-*bla* ECV304 cells were plated at 10,000 cells/well in a 384-well format in assay medium. They were stimulated with IL-1 in the presence of indicated amount of 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 Response Ratios for each DMSO concentration were plotted.