Validation & Assay Performance Summary

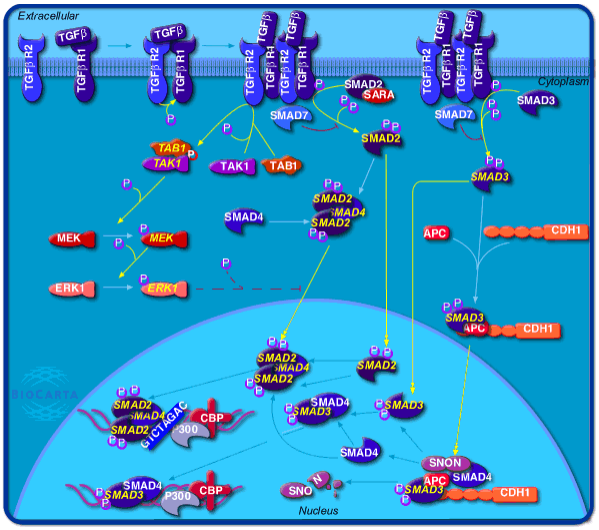
CellSensor® SBE-*bla* HEK 293T Cell Line

Cat. no. K1550

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

The Transforming Growth Factor beta (TGF-beta) signaling pathway is involved in cell growth and proliferation. The effects of TGF beta-1 varies due to cell type and other intracellular factors. In many cases it can inhibit the growth of a cell. This pathway utilizes the SMAD family of transcription factors. TGF beta-1 binds to its receptor on the cell surface. The receptors co-localize and activate SMAD2 and SMAD3. SMAD2 and SMAD3 can both bind SMAD4 along with additional proteins to influence transcriptional activity. The SMADs can bind a region of DNA called SBE (SMAD Binding Element).



Cell Line Description

The CellSensor® SBE-*bla* HEK293T cell line contains a beta-lactamase reporter gene under control of the SBE response element stably integrated into HEK293T cells. This cell line is a clonal population isolated in response to TGF beta-1 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 EC_{50} concentrations of TGF beta-1.

Validation Summary

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

1. **Primary agonist dose response under optimized conditions**

TGF beta-1 EC_{50}	= 0.403 ng/ml
Z'-Factor (EC_{100})	= 0.85
Response Ratio	= 4.3
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= <1%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 7 ng/ml

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

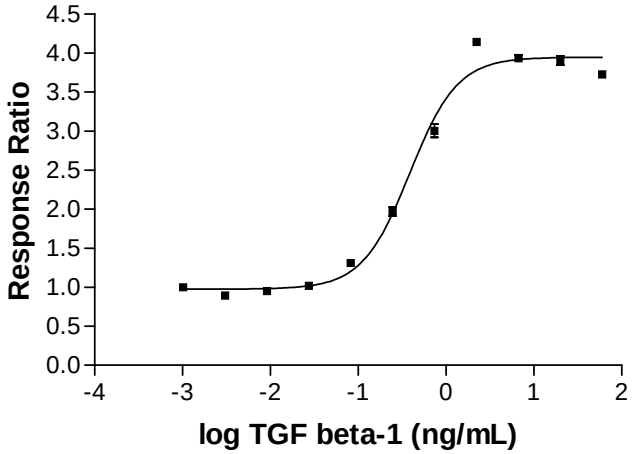
Assay Testing Summary

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

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

Primary Agonist Dose Response

Figure 1 –SBE-*bla* HEK293T dose response to TGF beta-1 under optimized conditions



SBE-*bla* HEK 293T cells (20,000 cells/well) were plated in a 384-well plate and stimulated with TGF beta-1 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 TGF beta-1 (data is average of data collected on three separate days).

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% CO_2 incubator. Maintain cells between 10% and 90% confluency. Do not allow cells to reach confluence

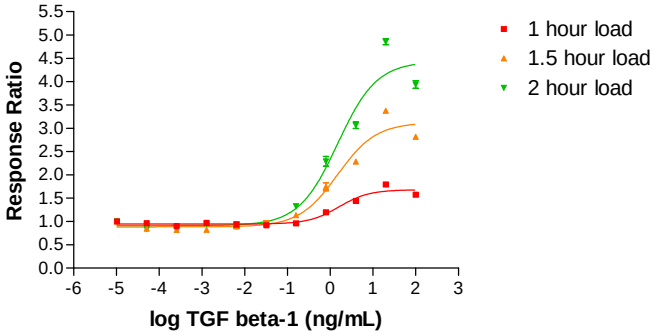
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. Freeze cells at 2 x 10^6 cells/ml in Freezing Medium. For optimal cell line performance, use Dialyzed FBS (Invitrogen # 26400-036). For detailed growth and maintenance directions, please refer to the protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium	Assay Medium	Freezing Medium
DMEM	90%	90%	—
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 (do not thaw with Blasticidin)	—	—
Cell Culture Freezing Medium	—	—	100%

Assay Performance with Variable Substrate Loading Time

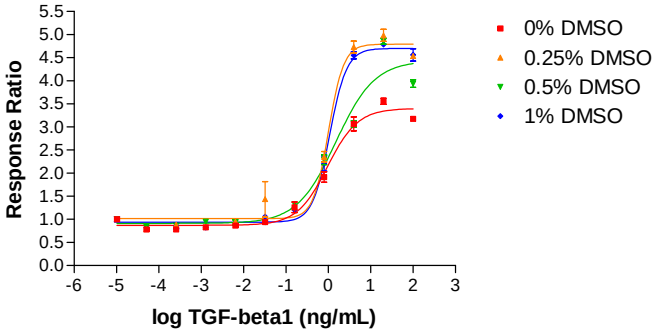
Figure 2 — SBE-*bla* HEK293T response to TGF beta-1 with 1, 1.5, and 2 hour substrate loading times



SBE-*bla* HEK293T cells were plated at 20,000 cells/well in a 384-well format. Cells were stimulated with TGF beta-1 at various concentrations 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 the indicated substrate loading times.

Assay Performance with Variable DMSO Concentration

Figure 3 – SBE-*bla* HEK293T response to TGF beta-1 using 0, 0.25, 0.5 and 1% DMSO



SBE-*bla* HEK293T cells (20,000 cells/well) were plated in a 384-well plate and treated with the indicated concentrations of TGF beta-1 with 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 TGF beta-1 concentration for each DMSO concentration.