

# Validation & Assay Performance Summary



## CellSensor<sup>®</sup> NFκB-*bla* HEK 293T Cell Line

Cat. no. K1664

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

Nuclear Factor Kappa B (NFκB) signaling regulates genes involved in apoptosis, viral defense, cancer, inflammation, and autoimmune disease. TNF alpha binds its receptor, which recruits a protein called TNF receptor death domain (TRADD). TRADD binds TNF receptor associated factor 2 (TRAF-2) which in turn activates NFκB inducible kinase (NIK). NIK phosphorylates proteins that inhibit NFκB in the cytoplasm, thereby marking these inhibitory factors for degradation. NFκB is then free to enter the nucleus and regulate transcription.

### Cell Line Description

The CellSensor<sup>®</sup> NFκB-*bla* HEK 293T cell line contains a beta-lactamase reporter gene under control of the Nuclear Factor Kappa Beta (NFκB) response element stably integrated into HEK 293T cells. This cell line validated for EC<sub>50</sub> and Z'-Factor under optimized conditions using Tumor Necrosis Factor Alpha (TNFα). This cell line has also been tested for assay performance under variable experimental conditions, including stimulation time, substrate loading time and DMSO concentration.

## 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)

TNF $\alpha$ EC <sub>50</sub>	= 0.7 ng/mL
Z'-Factor (EC <sub>100</sub> )	= 0.86
Response Ratio	= 22
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= up to 1%
Optimum Stim.Time	= 5 hours
Max. [Stimulation]	= ~50 ng/mL

### 2. Cell culture and maintenance

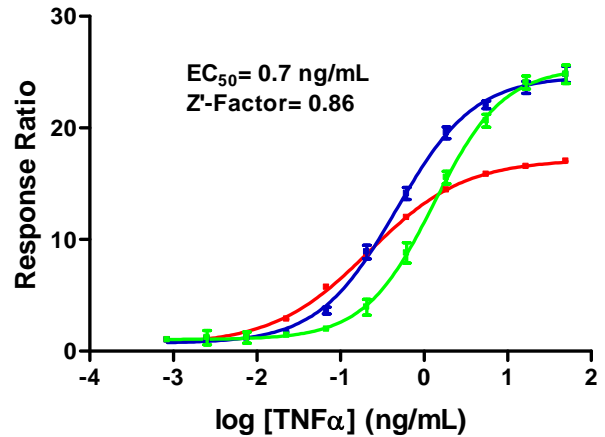
See *Cell Culture and Maintenance Section and Table 1*

## Assay Testing Summary

3. Assay performance with variable stimulation time
4. Assay performance with variable substrate loading time
5. Assay performance with variable DMSO concentration

## Primary Agonist Dose Response

**Figure 1** — NF $\kappa$ B-*bla* HEK 293T dose response to Tumor Necrosis Factor alpha (TNF $\alpha$ ) under optimized conditions



NF $\kappa$ B-*bla* HEK 293T cells (20,000 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 TNF $\alpha$  (BD Biosciences # 350466) 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 TNF $\alpha$  (n=16 for each data point).

## Cell Culture and Maintenance

Thaw cells in Growth Medium without Blasticidin and culture them in Growth Medium with Blasticidin. Passage or feed cells at least twice a week and maintain them in a 37°C/5% CO<sub>2</sub> incubator. Maintain cells between 10% and 90% confluency. Do not allow cells to reach confluence.

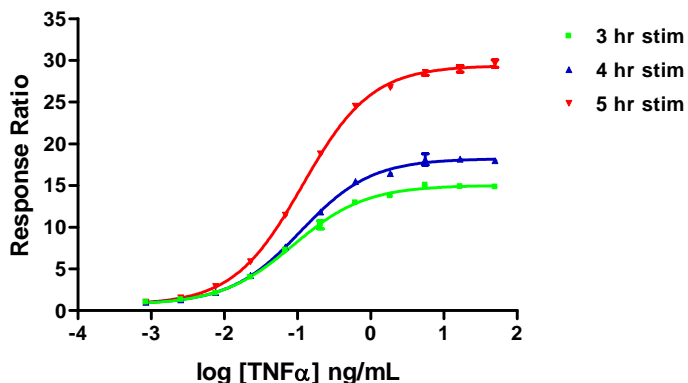
*Note:* We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For proper cell line performance, use dialyzed FBS (Invitrogen# 26400-036). For more detailed cell growth and maintenance directions, please refer to the protocol.

**Table 1 – Cell Culture and Maintenance**

Component	Growth Medium (+)	Growth Medium (-)	Assay Medium	Freezing Medium
DMEM w/ GlutaMAX™	90%	90%	90%	--
Dialyzed FBS <b>DO NOT SUBSTITUTE!</b>	10%	10%	10%	--
HEPES	25 mM	25 mM	25 mM	--
NEAA	0.1 mM	0.1 mM	0.1 mM	--
Penicillin	100 U/mL	--	100 U/mL	--
Streptomycin	100 µg/mL	--	100 µg/mL	--
Blasticidin	5 µg/mL	--	--	--
Recovery™ Cell Culture Freezing Medium	--	--	--	100%

## Assay Performance with Variable Stimulation Time

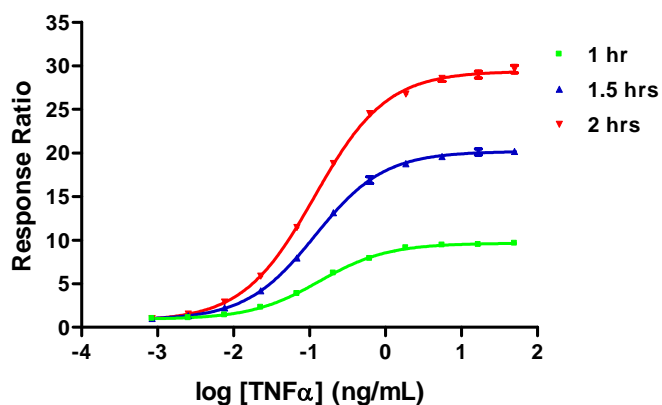
Figure 2 – NFκB-*bla* HEK 293T dose response to TNFα with 3, 4 and 5 hour stimulation times



NFκB-*bla* HEK 293T cells (20,000 cells/well) were plated the day of the assay in a 384-well assay plate. TNFα (BD Biosciences # 350466) was then added to the plate over the indicated concentration range. Plates were treated for 3, 4 or 5 hrs with TNFα 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 against the indicated concentrations of TNFα (n=8 for each data point).

## Assay Performance with Variable Substrate Loading Time

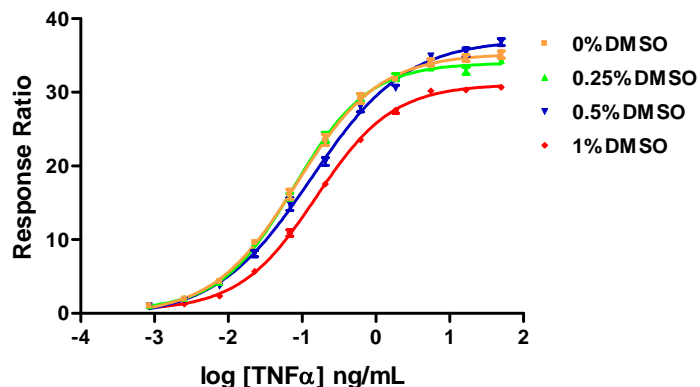
Figure 3 – NFκB-*bla* HEK 293T dose response to TNFα with 1, 1.5 and 2 hour substrate loading times



NFκB-*bla* HEK 293T cells were plated the day of the assay at 20,000 cells/well in a 384-well format. Cells were treated with TNFα (BD Biosciences # 350466) 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 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 against the indicated concentrations of TNFα (n=8 for each data point).

## Assay Performance with Variable [DMSO]

Figure 4 – NFκB-*bla* HEK 293T dose response to TNFα with 0, 0.25, 0.5 and 1% DMSO



NFκB-*bla* HEK 293T cells (20,000 cells/well) were plated the day of the assay in a 384-well assay plate. TNFα (BD Biosciences # 350466) was then added to the plate over the indicated concentration range with 0, 0.25, 0.5 or 1% final DMSO concentrations. Cells were 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 for each DMSO concentration were plotted against the indicated concentrations of TNFα (n=8 for each data point).