Validation & Assay Performance Summary

CellSensor® NFκB-*bla* THP-1 Cell Line

Cat. no. K1662

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 (NFKB) 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 NFKB inducible kinase (NIK). NIK phosporylates proteins that inhibit NFKB 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® NFκB-*bla* THP-1 cell line contains a beta-lactamase reporter gene under control of the Nuclear Factor Kappa Beta (NFκB) response element stably integrated into THP-1 cells. This cell line is validated for EC_{50} 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. Additional testing information using LPS is also provided.

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)**

TNFα EC_{50}	= 0.06 ng/mL
Z'-Factor (EC_{100})	= 0.90
Response Ratio	= 21
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= up to 1%
Optimum Stim.Time	= 5 hours
Max. [Stimulation]	= ~8 ng/mL

2. **Alternate Agonist Dose Response**
   LPS EC_{50} = 0.20 ng/ml

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

Assay Testing Summary

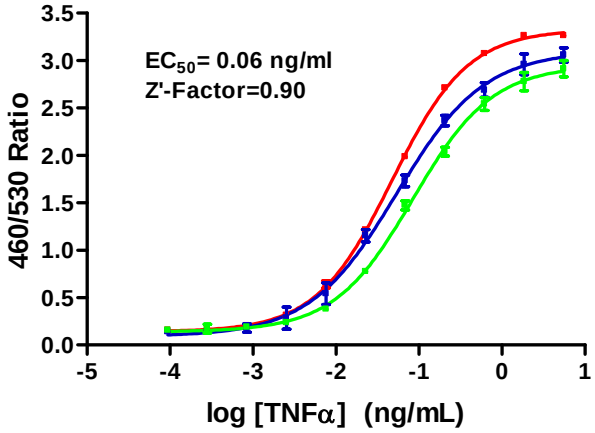
4. **Assay performance with variable stimulation time**

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

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

Primary Agonist Dose Response

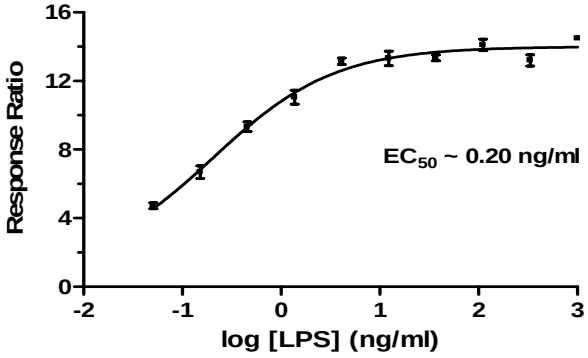
Figure 1 — NFκB-*bla* THP-1 dose response to Tumor Necrosis Factor alpha (TNFα) under optimized conditions



NFκB-*bla* THP-1 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 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 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 TNFα (n=16 for each data point).

Alternate Agonist Dose Response

Figure 2 — NFκB-*bla* THP-1 dose response to LPS



NFκB-*bla* THP-1 cells (20,000 cells/well) were plated the day of the assay in a 384-well format and stimulated with LPS 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 LPS (n=4 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_2 incubator. Maintain cells between $2x10^5$ and $2x10^6$ cells/ml. Do not allow cells to reach confluence.

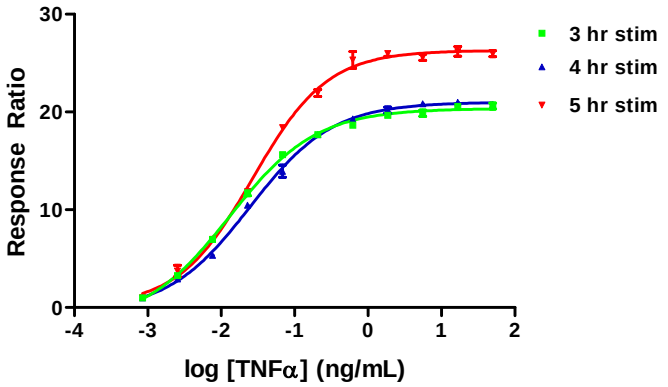
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For optimal cell line performance, use dialyzed FBS (Invitrogen# 26400-036). For more detailed cell growth and maintenance directions, please refer to the protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium (+)	Growth Medium (-)	Assay Medium	Freezing Medium
RPMI 1640	90%	90%	90%	80%
Dialyzed FBS **DO NOT SUBSTIUTE!**	10%	10%	10%	10%
NEAA	0.1 mM	0.1 mM	0.1 mM	0.1 mM
Sodium Pyruvate	1 mM	1 mM	1mM	1 mM
Penicillin	100 U/mL	100 U/ml	100 U/mL	--
Streptomycin	100 µg/mL	100 µg/mL	100 µg/mL	--
Blasticidin	5 µg/mL	--	--	--

Assay Performance with Variable Stimulation Time

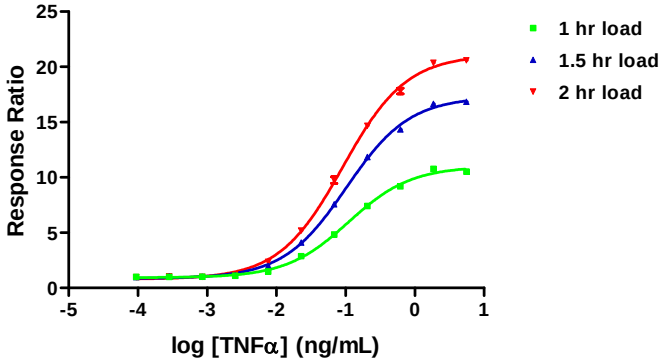
Figure 3 – NFκB-*bla* THP-1 dose response to TNFα with 3, 4 and 5 hour stimulation times



NFκB-*bla* THP-1 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

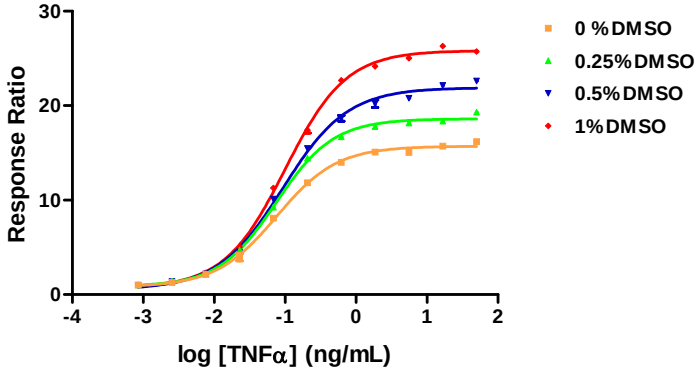
Figure 4 — NFκB-*bla* THP-1 dose response to TNFα with 1, 1.5 and 2 hour substrate loading times



NFκB-*bla* THP-1 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 5 – NFκB-*bla* THP-1 dose response to TNFα with 0, 0.25, 0.5 and 1% DMSO



NFκB-*bla* THP-1 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).